"AgNOR AS A TUMOUR MARKER AND ITS ROLE IN ASSESSING THE SEVERITY OF CERVICAL LESIONS"

Dissertation submitted

in partial fulfilment of the regulations for

the award of the Degree of

M.S DEGREE - BRANCH VI

OBSTETRICS AND GYNAECOLOGY

OCTOBER 2015

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

THE TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI,

TAMIL NADU.

CERTIFICATE

This is to certify that the Dissertation entitled **"AGNOR AS A TUMOUR MARKER AND ITS ROLE IN ASSESSING THE SEVERITY OF CERVICAL LESIONS"** submitted by **Dr. PRADEEPA. T,** appearing for M.S. Degree - Branch VI in Obstetrics and Gynaecology in October 2015 is a bonafide work done by her under my direct guidance and supervision in partial fulfilment of the regulations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai. I forward this to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, India.

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DECLARATION

I**, Dr. PRADEEPA. T,** solemnly declare that the Dissertation titled **"AGNOR AS A TUMOUR MARKER AND ITS ROLE IN ASSESSING THE SEVERITY OF CERVICAL LESIONS"** has been prepared by me under the expert guidance and supervision of **Prof. Dr. THAMILKOTHAI, MD (OG)** Professor, Department of Obstetrics and Gynaecology, Tirunelveli Medical College Hospital, Tirunelveli.

This dissertation is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the regulations for the award of MS Degree Branch VI (Obstetrics & Gynaecology).

It was not submitted to the award of any degree/diploma to any University either in part or in full previously.

Place: TIRUNELVELI

Date:

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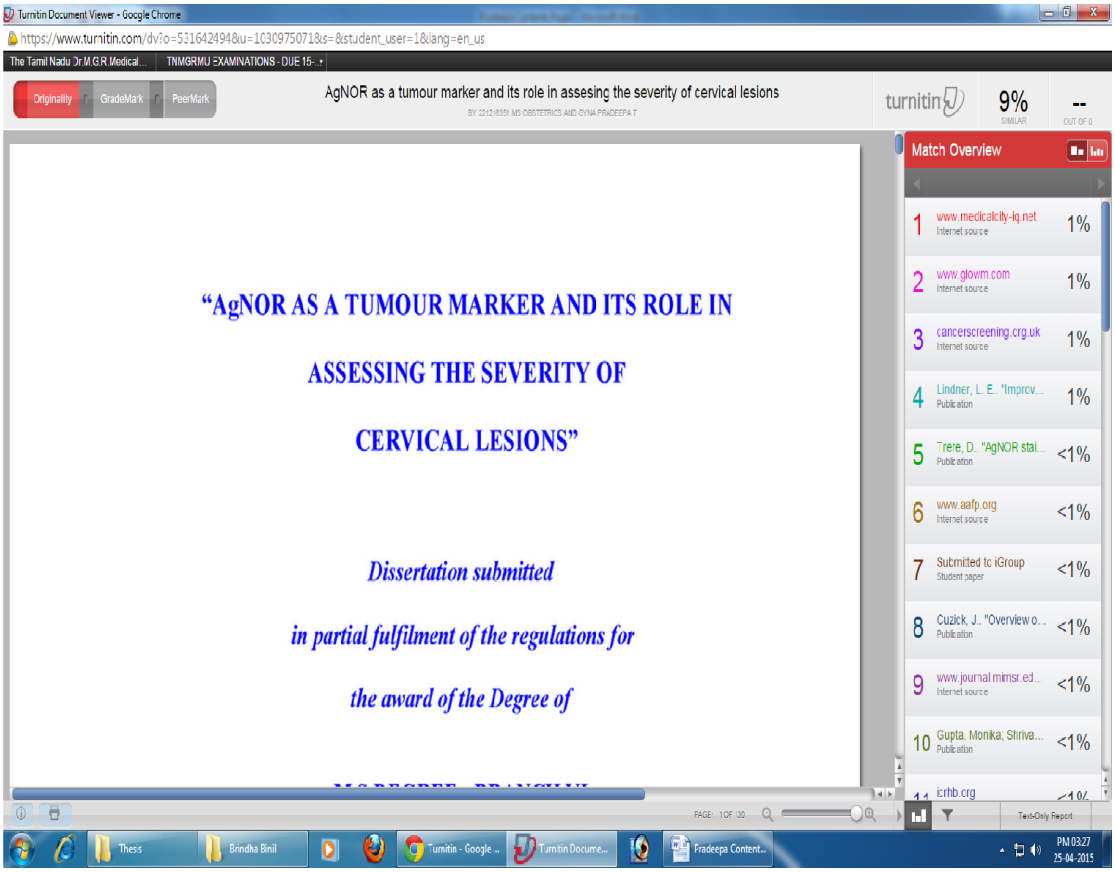
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CONTENTS

ABBREVIATIONS

AgNOR	-	Silver Stained Nucleolar Organizer Regions
HPV	-	Human Papilloma Virus
CIN	-	Cervical Intra epithelial Neoplasia
VIA	-	Visual Inspection with Acetic Acid
VILI	-	Visual Inspection with Lugol's Iodine

INTRODUCTION

Cervical cancer is the second most common cancer among females and is the first cause of cancer related deaths in developing countries. In urban areas, it accounts for 40% and 60% in rural areas.(cancer registry barshi) 1 in 154 deaths occur in the world due to carcinoma cervix(cancer statistics 2014). Key to prevent cervical cancer is awareness. Increased incidence in India due to lack of knowledge and it cannot happen to me attitude.

In those countries where effective screening, diagnosis and treatment is limited or absent, burden of cervical cancer is highest. Cervical cancer mostly hits the young population, whose lives seem shattered after they come to know of it.

1 out of 4 women die due to cervical cancer in the world is an Indian. New cases diagnosed in India per year is 1,40,000. More than 20 women die every day.8women die every hour. A women die every 7mins.(FERY ET AL IN GLOBOCAN 2008).

It is very important to find the effective screening method, so that the disease can be diagnosed earlier which helps to bring down the mortality and morbidity due to cervical cancer.

In developed countries, pap smear has brought down the incidence of cervical cancer. Better information can be provided by AgNOR, a molecular tumour marker which stands for silver stained (Ag) nucleolar organizer regions(NORs).

NORs denotes the loop of ribosomal DNA which transcribe to RNA. They are the tools used to study the proliferating capacity of the cell as they are related to cell cycle[1].Ionic silver precipitates at carboxyl and sulphydryl groups to form argyrophilic proteins. After silver staining NORs can be identified as black dots localised throughout the nucleolar area.

There are various number of clinical and histological parameters available for determining the aggressiveness of the tumour.DNA content, Ki-67, sphase fraction, oncogenes, AgNOR are some of the parameters. AgNOR is a simple, cost effective, quick and reliable adjuvant to histopathology.

AgNOR count was found to be more in malignant than in normal cells. It is used to deliniate the doubtful cases of CIN. It is also used as a prognostic tool.

Studies carried out in different tumour types demonstrated that malignant cells shows a greater protein than non malignant cells.

Study of AgNOR may become an important parameter to determine the aggressiveness of tumour at places where costly methods are not available.[2]

AgNOR size and number denotes the nucleolar and cell proliferative activity. Its distribution in the nucleus is useful in tumour detection and prognosis.Various studies are currently available to explore the possibility of finding out the tumour marker potential of AgNOR dots.There exists a relationship between AgNOR pleomorphism and neoplastic development in the uterine cervix.The correlation exists between HPV infection and AgNOR pleomorphism.AgNOR counts can replace HPV DNA testing to discover high risk SIL cases.(CANCER MEDICAL SCIENCE JOURNAL 2013).

AgNOR is able to pick up grey zones between benign and malignant. Eventhough pap smear is the universal test for screening,single conventional pap smear is unable to detect 40-50%of biopsy confirmed HSIL and invasive cervical cancers. AgNOR count is useful in doubtful cases, especially when cervical smears shows inflammatory features or metaplasia.(Lynette denny BJOG 2005).

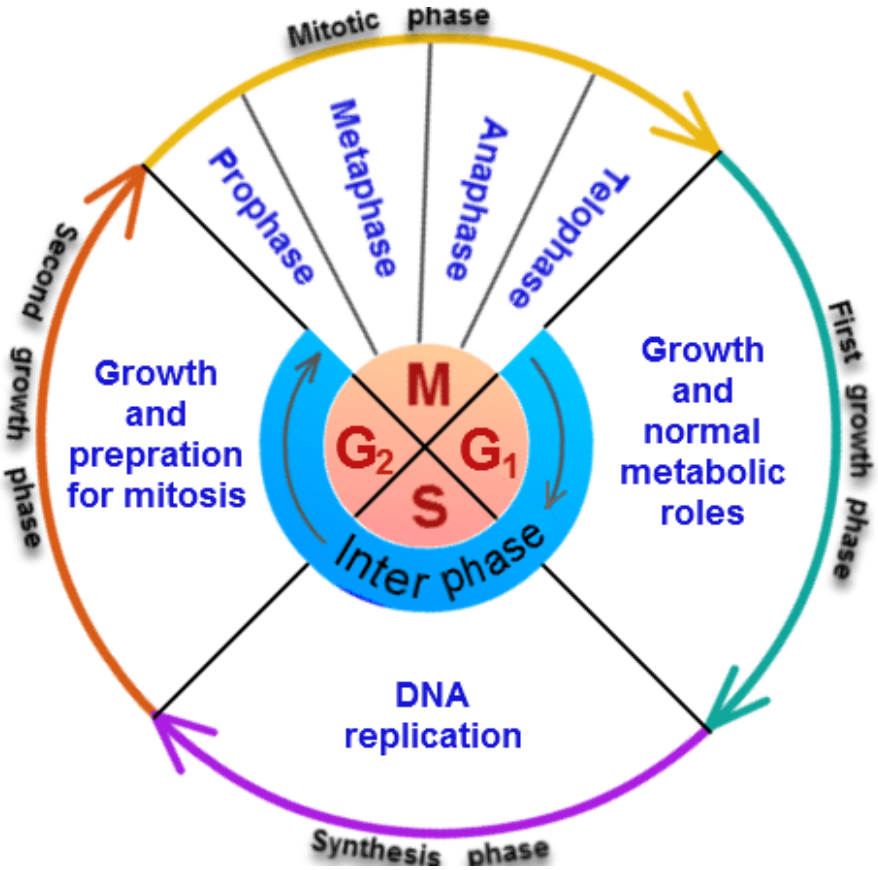
In this study, AgNOR counts of cervical lesions are compared with those of HPE and results are analyzed for statistically significant difference in different groups.

AIM OF STUDY

1. Women with abnormal ymptoms and abnormal cervix are subjected to cervical biopsy and histopathological diagnosis done with H & E stain and AgNOR scoring with silver stain.
2. To correlate histopathological results and AgNOR counts of cervical lesions.

REVIEW OF LITERATURE

- Cell cycle is the ordered set of events resulting in cell growth and its division.
- G0- This is the phase where a cell quits dividing and it is the temporary resting period.
- G1-In this phase, cells increase in size, produce RNA and synthesise protein. This ensures that everything is ready for DNA synthesis.
- S-DNA replication occurs in this phase. The complete DNA instructions in the cell must be duplicated to produce two similar daughter cells.
- G2-In this phase, cell continue to grow and produce new proteins.
- M-Cell growth stop at this stage. Cell is orderly divided into two similar daughter cells
- NORs located on the short arm of chromosomes 13,14,15,21,22[3,4]
- NORs are clearly demonstrated in metaphase.
- DNA content at the end of S phase is an indicator of proliferative activity. AgNOR detects the DNA content at this stage.
- AgNORs is known to increase with increase in cell ploidy, transcriptional activity and in states of active cell proliferation.



Stages of Cell Cycle

- Transition from one phase to another regulated by different cellular proteins.they are the cyclins and cyclin dependent kinases.cyclins are required to end mitosis in a cell cycle.
- AgNORs in oncology workshop held in Berlin in 1993 first identified the guidelines for AgNOR protein evaluation.
- Interest in AgNOR proteins increased greatly around the end of 1980s. (DEREZINI ET AL 1994).

AgNOR IN ONCOLOGY:

Guski et al proved that AgNOR count among the malignant cases is significantly higher than benign .They demonstrated that total AgNOR number and area increase from atypical ductal hyperplasia to ductal carcinoma in situ in breast cancer.[5]

Rivera et al analyzed the number and distribution pattern of NORs by silver staining in the samples that combined a model of chemical carcinogenesis and chronic stress[6]

AgNOR is used as a major and independent prognostic factor in surgically treated colorectal cancer. This study emphasized the efficiency of the predictive power of AgNOR with regard to chemotherapy[7]

Gupta et al in 2013 demonstrated that AgNOR parameters are useful indicators to evaluate the malignant behaviour of Gall bladder carcinoma. AgNOR predicts the response of the tumour to treatment and detects residual viable tumour.[8]Nishizawa et al reported that an increased number and size of AgNOR dots associated with lymphnode metastasis and shorter survival of patients who had undergone surgical resection of gall bladder cancers.[9]

AgNOR technique is an efficient and practical tool to distinguish mesothelium from neoplastic cells. It is also useful as a prognostic markers of survival in squamous cell carcinoma of lung. Bernardi et al are interested to determine the prognostic markers from routine histopathological and cytological material by H&E and AgNOR stained preparations.[10]

AgNOR count is used as an adjunct and provides a significant cell kinetic evaluation of prognostic lesions. Nakamura et al analyzed by his study that there occurs a statistically significant difference between benign and malignant lesions in AgNOR count.[11]

Pollock studied the correlation of NORs in exocrine pancreas, chronic pancreatitis and adenocarcinoma. It is definitely useful in differentiating various pancreatic lesions.[12]

Mulazim demonstrated that AgNOR count,its size and dispersion was proved to be an important marker in grading astrocytic lesions.[13]

Yamanoto studied AgNOR in Renal cell carcinoma and its efficacy as a marker of proliferative activity.AgNOR values were found to be higher than those in normal tubules and NORs increased with the progress of grades.

Abbasi et al studied the importance of AgNOR in differentiating keratoacanthoma from squamous cell carcinoma.[14]

AgNORs are also useful as a proliferative tumour marker in desmoid tumour.[15]

AgNOR in cervical lesions:

Kaushik et al conducted a study to assess the utility of AgNOR counts in differentiating cervical lesions.Mean AgNOR counts showed a statistically significant increase from normal to chronic cervicitis to CIN 1,2&3.This study proved that AgNOR count is an useful adjunct to routine histopathology to evaluate cervical lesions[16].

Srivatsava et al investigated regarding the usefulness of AgNOR pleomorphism to assess the progression of cancer cervix. They have found that the pleomorphism of AgNOR increases with progression of cervical lesions and highest number is seen in squamous cell carcinoma[17].

Alarcon Romero et al [18] reported that significant difference was found with various types of AgNOR dots with infection of high risk HPV types. They concluded that study of HPV types and polymorphism of AgNOR can be useful as prognostic factors to estimate the progression of cervical lesions.

Terlikowski et al conducted a study to correlate between the number of AgNOR granules and degree of CIN. Number of cells with 1 dot decrease with increasing grade and number of cells with 4 or more increased with increasing grade of cervical lesions.[19]

Misra Js et al studied 50 cervical smears and found an increase in AgNOR count with progression of cervical lesions.[20]

AgNORs in cervical cytology is an adjunctive tool to PAP stained smear and help in finalising the diagnosis.Shukla et al emphasis the fact that the AgNORs can be declared as a definitive index for assessing the cell proliferative activity.[21]

Ritu et al employed AgNOR staining in 112 female patients and observed that there is a significant increase of AgNOR count in malignant lesions than in premalignant and benign conditions.[22]

Vijaya et al compared the various proliferative and apoptotic indices in different cervical lesions and concluded that AgNOR counts are useful cell kinetic analyzer and aid in patient management decisions. In this study maximum counts were obtained in invasive carcinoma[23]

PREINVASIVE AND INVASIVE LESIONS OF CERVIX

Cancer cervix has been the most important cancer in India.Mortality from cervical cancer is expected to increase to 79% from 74,118 to 1,32,745 deaths by 2025.(NATIONAL CANCER REGISTRY PROGRAMME 2009).

Incidence of cervical dysplasia is 15:1000 in women who were cytologically screened and severe dysplasia is reported to be 5:1000. In Tamilnadu ,Chennai had the highest incidence rate of cervical cancer among population based cancer registries.

Cervical cancer is curable and preventable by detecting it in an early stage(WHO,2006).5yr survival rate for the women when detected at earliest stage is 92% and for all stages is 71%(American cancer society 2009).women can develop CIN at any age, however women generally develop it between the ages of 25-35yrs.2,50,000-1 million women were diagnosed with cervical dysplasia in developed countries(Stanley et al JAN 2013).

As cervix can be easily accessible, cancer cervix can be diagnosed even in its preinvasive stage. If it is treated in early stages, the patient can be cured of the disease.

ANATOMY OF CERVIX:

Cervix is spindle shaped and measures about 2.5cm.mucosal lining of cervix differs from the body of uterus by the absence of submucosa.cervix contains more of fibrous tissue and collagen than muscle fibers.

ENDOCERVIX:

It extends from internal os to ectocervix. It contains the endocervical canal and lined by columnar epithelium which is thrown into folds and project into the underlying stroma forming cypts.

FUNCTIONS:

1. Cilia are directed downwards and it prevents ascending infection
2. Cells sieve out abnormal sperms and it provides nutrition for healthy sperms.
3. It allows capacitation of sperms.

ECTOCERVIX:

It extends from SCJ to vaginal fornices. It is hormone sensitive and covered by non keratinising stratified squamous epithelium.

SQUAMO COLUMNAR JUNCTION:

It is the most important junction where the squamous epithelium merges with columnar epithelium of ectocervix. Around 1-10mm.Here the cells are sensitive to irritants, mutagens and HPV 16,18.

It is the dynamic point that changes throughout life as a result of metaplastic changes in the columnar epithelium of cervix. The reserve cells lying beneath the columnar epithelium at this junction sometimes transforms into mature squamous cells known as metaplasia. Metaplastic cells are normal cells without nuclear atypia and do not become malignant. Atypical metaplasia with abnormal nuclear changes are the precursors of dysplasia and malignancy.

Transition in SCJ occurs in various phases of life:

1. Prepubertal period:

   Columnar epithelium spreads over the external os

2. Puberty:

   Metaplasia of columnar epithelium under the influence of estrogen brings the squamous epithelium close to external os.

3. Premenopausal:

   It gets drawn into external os.

4. Pregnancy:

   Pouts out of os.

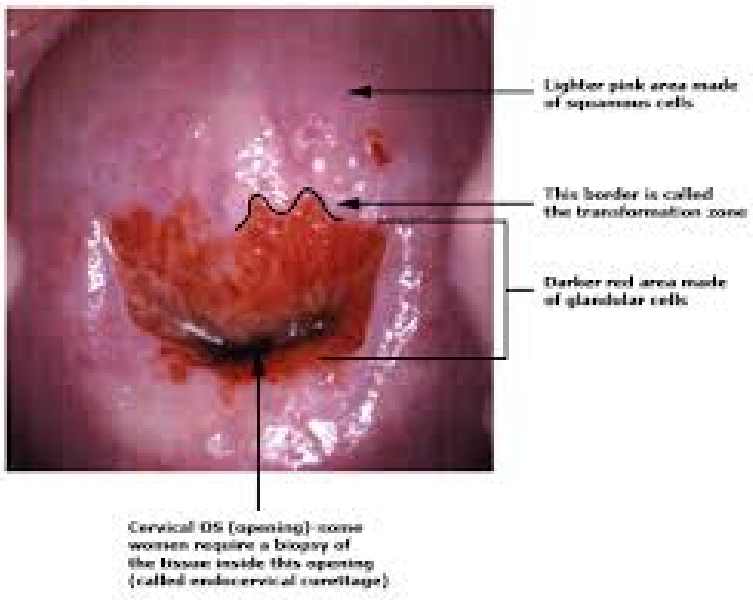
TRANSFORMATION ZONE:

Metaplasia advances from original SCJ inwards towards the external os and this process establishes an area called transformation zone.it is the zone between original SCJ to physiologically active SCJ.

It is visible as a distinct white line after the application 3-5% acetic acid. Transformation zone is the site of origin for 90% of precancerous and cancerous lesions.

Reproductive age group women shows more active metaplasia than menopausal women and hence they are at low risk.

SCJ is well outside the external os during reproductive period and this area can be screened for malignancy.



AETIOLOGY AND RISK FACTORS

1.BIOLOGICAL RISK FACTORS:

1. Herpes simplex virus 2
2. Chlamydia trachomatis
3. HIV

Herpes simplex virus 2:

Seroprevalence was higher in a study than in control subjects conducted by smith et al and association was consistent in squamous cell carcinoma and adenocarcinoma.[25]

Chlamydia trachomatis:

Chlamydia trachomatis serum antibodies increases the risk of cervical cancer by 1.8 fold in all countries. The study suggests the possibility of Chlamydia trachomatis increases invasive carcinoma after accounting for HPV infection.[26]

HIV:

Ahdieh et al [27] identified a higher incidence of cervical abnormalities among HIV infected women(13.4%) when compared to HIV negative women (2.4%)HPV and HIV share behavioural traits that define the high risk group.progression of cervical lesions is related to severity of immunosupression as indicated by CD4 counts.

BEHAVIOURAL RISK FACTORS:

1. Smoking:

Nicotine and tobacco are the specific carcinogens detected in cervical mucus of smokers.it indicates tha there exists a synergistic relationship between cigarette smoking and HPV for development of dysplasia and carcinoma.[28]

2.Coitus before the age of 18yrs

3.Multiple sexual partners

4.Multiparity

5.Poor personal hygiene

6.Infrequent or absent cancer screening pap tests

7. Dietary:

At present, none of the cohort studies asses suspected nutritional or dietary factor controlling HPV infection. Further studies are needed with long follow up periods.

MEDICAL RISK FACTORS:

ORAL CONTRACEPTIVE PILLS:

A recent meta analysis on the association between OCPs and cervical cancer establishes the fact that there exists a linear dose response

relationship between years of use and risk of cervical neoplasia.Duration of risk after OCP cessation is yet to be determined.[29]

2.IMMUNOSUPRESSION

DEMOGRAPHIC RISK FACTORS:

1. Ethnicity
2. Low socioeconomic countries
3. Age

HUMAN PAPILOMA VIRUS AND CERVIVAL CANCER:

HPV genomes are found in all stages of cervical cancer.[30] HPV infection is the initiating agent and is the primary cause of cervical cancer. [31] portions of HPV DNA integrates into host cell. This integration appears to be essential for malignant transformation which requires expression of E6 & E7 oncoproteins [32]

Most women with cervical neoplasia expresses HPV DNA. More than 120 types of HPV DNA have been identified. Of these 120,30 types infects squamous epithelium of lower anogenital tract of men and women.

90% of CIN is attributed to HPV infection. Type 16 is most commonly found in invasive cancer,CIN2 and CIN3.[33] 47% of women with cancer cervix in all stages infected with HPV 16.[34] 23% of women

with invasive cancers.5% of women with CIN2 &CIN3,<2% with negative findings are affected with HPV 18 [35].Thus HPV18 is more specific than HPV 16.

Most of the time HPV infection does not persist. Those that persists may remain latent for many years. Most of the women have no clinical evidence of disease and the infection is suppressed or eliminated [36]. Others exhibit low grade cervical lesions that regresses spontaneously. Most probably the infection will clear in 9-15 months.[37].Average time between HPV infection and pre cancer is about 7-10 years. Only the minor population develops persistent infection that may progress to CIN. Persistent HPV infection increases the risk of high grade lesions and is required for the development and maintenance of CIN.

HPV VACCINE:

Development of HPV vaccine is an important step in primary prevention.

GARDASIL is a quadrivalent vaccine containing virus like particles for HPV 6,11,16,18 approved in 2006. CERVARIX is a bivalent vaccine containing HPV 16 & 18.For the women who are seronegative and HPV DNA negative for HPV 16 & 18 at vaccination and received all

the three doses, vaccine is 100% effective. This protection lasts for 6.4 yrs after vaccination.[38]Women who are seropositive,but DNA negative efficacy is 100%[39]Women who are HPV 16 & 18 DNA positive, vaccine donot show efficacy. These vaccines are not able to clear an active infection and are used to prevent CIN, not used to treat it.

HPV vaccine cross protects from other high risk types. Bivalent vaccine protects from persistent infection with HPV types 31,33 & 45 [40]. Quadrivalent vaccine shows cross protection against CIN2 and HPV 31 [41]. Follow up for three years indicates that both vaccine reduces the referrals to colposcopy by 26% and 20% respectively [42]. It reduces the excisional procedures by 69% and 42% respectively.

Quadrivalent vaccine prevents genital warts caused by HPV 6 & 11.This vaccine gives 89% protection in men and boys against HPV infection. Hence this vaccine is approved for males.

Women who were HPV negative at vaccination had 100% reduction in vaginal and vulvar intraepithelial neoplasia. The vaccinated women shows only50% reduction in VIN AND VAIN. This data reveals that HPV types other than 16 & 18 are responsible for a significant proportion of VIN & VAIN. So women who are already positive for HPV 16 & 18 are not protected from VIN &VAIN.

To improve the significant antibody response to the antigen,it is combined with adjuvant.In quadrivalent vaccine,the adjuvant is aluminium hydroxyphosphate sulphate.In bivalent vaccine,the adjuvant is aluminium hydroxide combined with monophosphoryl lipid A.This provides a link between HPV and activation of innate immune system and it provides higher antibody titers than aluminium adjuvant [45].

Whether booster dose is needed after vaccination is not yet approved as most of the women at their twenties get exposed to HPV at the maximum. As first vaccination is given less than 10 years, it is advisable to give booster, but not yet proved as the studies are ongoing.

In 2007, American cancer society issued a guidelines. According to age, HPV vaccine may be provided as early as 9 years and as late as 18 years. For young women between 19-26, there are insufficient data .Screening practices for cervical cancer and dysplasia should remain unchanged for both vaccinated and unvaccinated women.

PREMALIGNANT AND MALIGNANT LESIONS OF CERVIX

CIN –It is the histopathological terminology in which part or full thickness of stratified squamous epithelium is replaced by cells showing varying degrees of dysplasia. Basement membrane however is intact.

Dysplasia refers to alteration in cell size,shape and polarity.The term dysplasia was first introduced by Reagen in 1953.

There is an alteration in nucleolar cytoplasmic ratio and hyperchromatic nuclei with marginal condensation of chromatin and mitotic f igures.This progresses and ends up in invasive cancers.

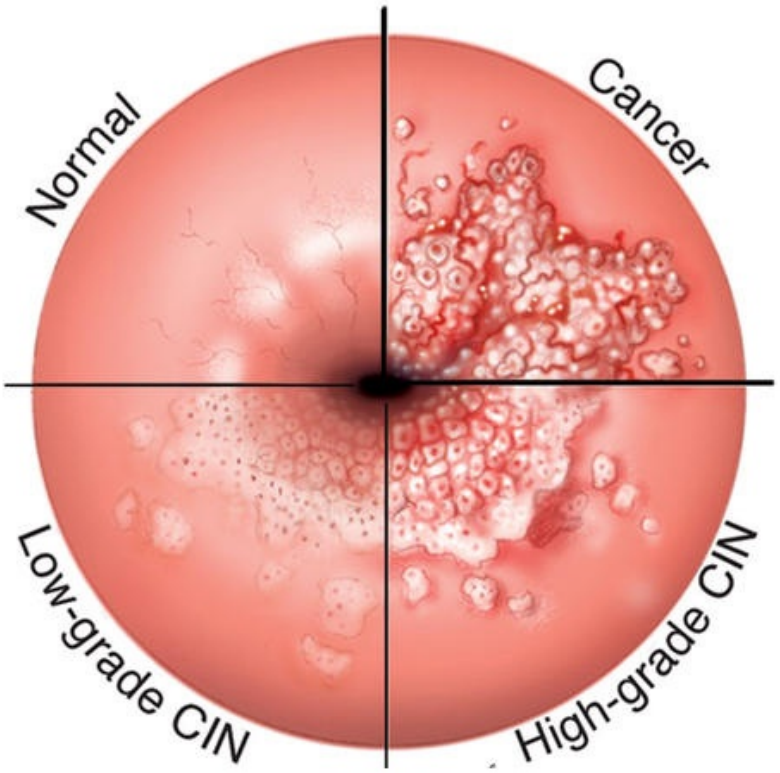
According to woodruff and novak(1979),three degrees of dysplasia:

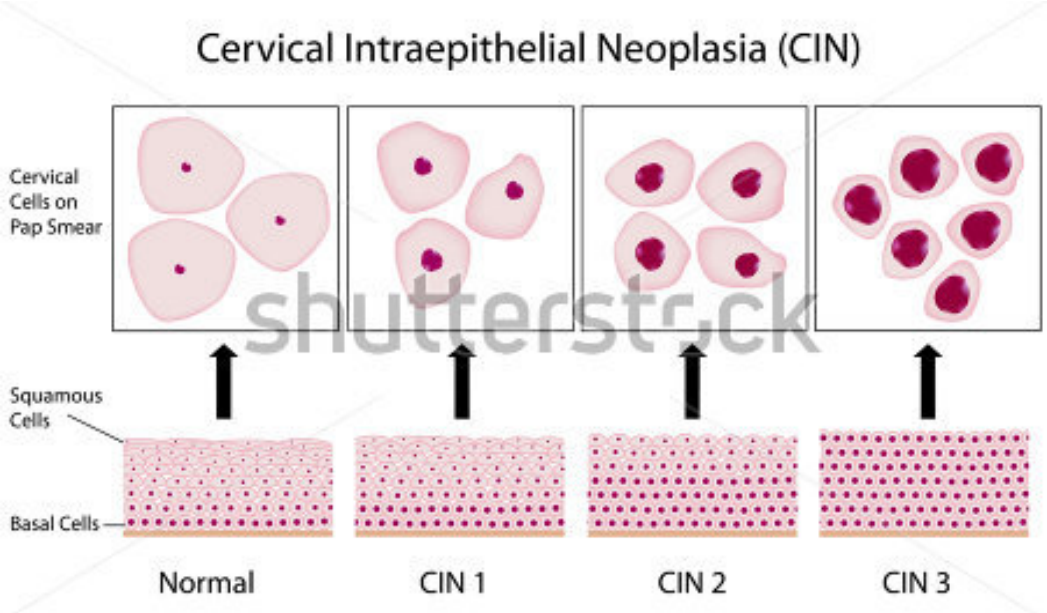
MILD:

- Dysplastic cells are confined to lower one third of epithelium.
- It may be due to infection in young women indulging in sexual activity.

MODERATE:

- Dysplatic cells confined to lower 50-75% of epithelium.
- Mostly the cells are intermediate type


Normal
Cancer
Low-grade CIN
High-grade CIN


Cervical Intraepithelial Neoplasia (CIN)
Cervical Cells on Pap Smear
Squamous Cells
Basal Cells
Normal
CIN 1
CIN 2
CIN 3

SEVERE:

- Entire thickness of epithelium replaced by dysplatic cells.
- Cornification and stratification is lost.
- Basement membrane intact.
- No stromal infiltration.
- Cells are mostly parabasal.

ICMR reports an incidence of dysplasia to be 15:1000 women who are cytologically screened [shaw]. Richart in 1966 first introduced the term CIN to denote intraepithelial abnormalities of varying degrees.

FEATURE VARIATIONS WITH INCREASING SEVERITY OF DYSPLASIA :

DECREASE	INCREASE	VARIES
Amount of cytoplasm	N/C ratio	Hyperchromatic nuclei
Cellular cohesion	Mitosis	Anisokaryosis
Multinucleation	Anisochromatism	Nuclear hypertrophy
Degree of maturation	Nuclear membrane	
Normal flora	irregularities	

PROGRESSION AND REGRESSION OF CIN:

Cin	Regress	Persist	Progress to CIN3	Progress to Carcinoma
CIN 1	57%	32%	11%	1%
CIN2	43%	35%	22%	5%
CIN3	32%	56%	-	>12%

(FROM OSTOR AG – INTERNATIONAL GYNAECOLOGY PATHOLOGY 1993;12)

SCREENING PROCEDURES

1. PAP TEST:

Screening for cervical cancer precursors using exfoliative cervico vaginal cytology, the pap test was successful in reducing the incidence of cervical cancer by 79% and mortality by 70% since 1950[46]

Cytology based screening program can be implemented effectively only if infrastructure and lab quality assurance are consistently met. Various studies have demonstrated limited sensitivity of conventional pap cytology ranging between 30-87%This is an area of major concern as high false negative rates results in premalignant and malignant cells being misdiagnosed as normal. Consequently, the test needs to be repeated at frequent to achieve programme effectiveness.pap test has repeatedly demonstrated good specificity ranging from 86-100%.

Pap smear should be obtained prior to vaginal examination by placing the patient in dorsal position using cuscos speculum after visualising SCJ. Cervix is exposed and SCJ is scraped by Ayre's spatula around 360 degree.

Scrapings spreaded to glass slide and fixed by using 95% ethyl alcohol and ether. Fix for 30 minutes, air dried and then stained. conventional pap test technique needs to be improved in order to reduce

false negative errors. Sampling errors occurs because a lesion is too small to exfoliate cells or the device did not pick up the cells and transfer them to the fixation media. To overcome this, several new technologies like LBC and automated pap smears are being explored.

DRAW BACKS OF PAP SMEAR:

SENSITIVITY	:	not very high
YIELD OF CELLS	:	20%
ERRORS	:	in sampling

Interpretation		detection
Low false negative rate	:	10%-29%
High specificity	:	97-100%
Low sensitivity	:	29-56%

LIMITATIONS OF PAP SMEAR:

1. Complex laboratory test.
2. Requires cyto-technician for reading and pathologist for review.
3. Continuous monitoring needed to maintain high quality results.
4. Reports often take minimum of 1-2wks.
5. Follow up of women is difficult.
6. Usually available in large cities in many countries.

LIQUID BASED CYTOLOGY:

Cells are collected using a very small brush that is broken off into a small pot containing preservative solution. In the cytology lab, sample is filtered or centrifuged to remove excess blood and debris and the cells are then transferred to the slide in a monolayer. It interprets at higher speed, lower rate of unsatisfactory smears and possibility of ancillary molecular testing using remnant fluid. More expensive and requires sophisticated equipment.

Automated pap smears, the material on the slide is reviewed and scored based on algorithm. This includes variety of visual characteristics such as shape and optical density of cells as to the likelihood of an abnormality being present. Auto pap selects a sample of slides for manual re-screening that are enriched with abnormalities. Manual review is undertaken for cases which is designated by either the cytologist or the complete ranking as abnormal.

CLASSIFICATION OF PAP SMEAR :

Pap Class System (1954)	Scheme 1 Reagen (1956)(WHO)	Scheme 11 Ruchart (2001)	Scheme 111 Bethesda (1988)
CLASS1 CLASS2 CLASS3 CLASS4 CLASS 5	Negative for malignant cells Koilocytes Mild dysplasia Moderate dysplasia Severe dysplasia Carcinoma in situ Invasive cancer	Negative CIN-1 CIN-2 CIN-3 INVASIVE CANCER	Within normal limits Reactive and reparative changes(ASCUS) LSIL HSIL Invasive cancer

HPV BASED SCREENING TEST:

Possible clinical applications of detecting high risk HPV DNA are

1. As a primary screening test, solely or in combination with cytology to detect cervical cancer precursors.
2. As triage for women with cytology findings of ASCUS or LSIL in order to select women who need referral for colposcopic diagnosis and treatment.

3. In subsequent management of women referred for colposcopy due to abnormal smears but where findings on colposcopy/biopsy are negative.
4. As a follow up test for women treated for high grade CIN with local ablative or excisional therapy in order to identify and accurately evaluate the treatment outcomes.

Hybrid capture technology is the most commonly used test.HPV DNA testing is more sensitive but less specific than cytology in detecting high grade lesions. Lower specificity is due to presence of infection without any cytological changes.

HPV DNA test which is more sensitive should be applied first followed by cytology which is more specific to determine the management.HPV negativity or a combination of HPV and negative cytology denotes a longer disease free interval against CIN2.

Follow up results of a RCT involving single round screening in low resource settings demonstrates reduction in numbers and deaths from cervical cancer with HPV testing.

Investigators found that HPV testing to be most objective and reproducible of all cervical screening tests. It is less demanding in terms of quality assurance and training. With the availability of simple,

affordable and accurate HPV test, this can be used as a primary screening in low resource settings for women of atleast 30yrs of age.[47]

Care HPV is a new test which is very simple and can be performed even by health care workers. Evaluation of clinical accuracy of care HPV showed it to be substantially better than visual inspection methods with sensitivity and specificity of 90%&84.2%respectively on specimens collected by nurse midwife.[48]

VISUAL INSPECTION METHODS:

In developing countries, it has been difficult to maintain effective cervical cytology programs due to lack of resources, trained man power, infrastructure and requirement of multiple visits.

Low cost alternative method is visual inspection of cervix with VIA/VILI. Medical as well as paramedical staff can be easily trained. Application of 3-5% dilute acetic acid on the cervix during VIA results in reversible coagulation of cellular proteins. The areas with dysplasia or invasive cancer undergoes maximal coagulation due to large number of undifferentiated cells in the epithelium that have a reversal of N/C ratio. Therefore these areas appears acetowhite.

During VILI, normal squamous epithelium containing glycogen takes up iodine staining mahogany brown or black. The precancerous

cells and invasive cancer do not take up iodine due to lack of glycogen and appear as well defined thick mustard or saffron yellow areas.

Sensitivity and specificity in a pooled analysis of eleven cross sectional studies were 77% and 86% for VIA,91% and 85% for VILI respectively.[49]

4.COLPOSCOPY:

- Colposcope is a binocular microscope. Useful in locating abnormal areas and directing biopsy so the need for conisation is reduced.
- To study the cervix when pap smear detects abnormal cells.
- To locate the abnormal areas and take a biopsy.
- To study the extent of abnormal lesion.
- Conservative surgery under colposcopic guidance.
- Follow up of conservative therapy cases.
- Abnormal areas are acetowhite areas, punctuation, mosaic pattern and atypical vessel pattern.
- Punctuation referred to as dilated capillaries terminating on the surface appear from the ends as a collection of dots.
- Mosaics are the terminal capillaries surrounding roughly circular or polygonal shaped blocks of acetowhite epithelium.
- Atypical vessel pattern seen in invasive cancer and it includes branching,reticular and looped vessels.

CERVICAL BIOPSY:

It is the gold standard confirmatory test. Punch biopsy, conisation biopsy and loop excision biopsy are the different types of biopsy methods done. Punch biopsy is the most common method practiced but it does not include stroma.

Cone biopsy is both diagnostic and therapeutic. It causes bleeding, infection, cervical stenosis and cervical incompetence.

AgNOR COUNT:

It is a new molecular tumour marker which stands for silver stained nucleolar organizer regions.

ADVANTAGES:

1. Low cost
2. It can be used in cytological smears and tumour material
3. Does not require fixatives.

DISADVANTAGES:

1. Counting procedures are manual and hence long and tedious
2. Sometimes inaccurate due to observer error
3. Overlap and coalescence may result in misjudged counts as denoted by crocker et al.

SCREENING INTERVALS

DEVELOPED COUNTRIES:

ACOG GUIDELINES:

- Initial screening : At the age of 21 or 3yrs after vaginal sex.
- Interval between screening: Every year for either liquid based pap or conventional smear. Every 2-3 year after age 30 with three consecutive normals.
- More frequent screening for HIV positive women (twice for first year and annually after).
- Discontinuation for screening is reasonable between 65 to 70 yrs, with reassessment of risk factors annually.
- In the setting of posthysterectony for benign indications, it is reasonable to discontinue screening in the absence of history of high grade CIN or cancer.

DEVELOPING COUNTRIES:

In low resource settings, where screening programmes are organised less effectively, single or two screening in lifetime may be the only option. Greatest public impact is achieved by screening women between 30-39yrs of age.

Screening once at 35yrs with VIA or HPV DNA leads to reduction in lifetime risk of cervical cancer by 25-36%.screening twice (at 35 & 40 yrs of age) provides life time reduction risk by 40% [50]

Choosing a suitable screening test with good efficacy and one which is feasible, affordable for implementation with respect to available technical expertise and man power is an important aspect of a screening programme.

It is important to identify the factors which determine the participation of women, incorporating a comprehensive health education programme prior to screening, personal invitations, proximity of clinics to the target women all help in increasing compliance.

MANAGEMENT OF CIN

TREATMENT MODALITIES:

1. Ablation Techniques
2. Excision Techniques

ABLATION TECHNIQUES:

1. Cryosurgery
2. CO2 laser vapourization

CRYOSURGERY:

It destroys the epithelium of cervix by causing destruction of the cell by crystallizing intracellular water. Temperature required is -20 to -30 degree celcius.NO2 and CO2 are the most commonly used gases for this purpose.

Use of double freeze technique with 3min interval each of freeze thaw freeze improves the efficacy. Probe should cover the entire lesion and have to create a 4-5mm ice ball beyond the edge of the probe. The women should abstain from intercourse for 4weeks.

ADVANTAGES:

1. Outpatient procedure
2. Low cost
3. No bleeding

DISADVANTAGES:

1. No tissue specimen for HPE
2. Profuse white discharge
3. Cephalad migration of SCJ

Townsend showed that cure rates are related to the size of the lesion. positive findings on ECC causes reduction in cure rate. Endocervical gland involvement also causes failure rate of 27% compared to 9% those who did not have much involvement. [51]

CRITERIA FOR CRYOTHERAPY:

1. The entire lesion is located in the ectocervix without extension to the vagina.
2. The lesion is visible in its entire extent and does not extend more than 2-3mm into the canal.
3. CIN is confirmed by cervical biopsy/colposcopy.
4. There is no evidence of invasive cancer
5. The women is not pregnant.
6. There is no evidence of pelvic inflammatory disease.
7. The women has given informed written consent to have the treatment.

LASER ABLATION:

This procedure gained popularity in the mid 1980s.It is also performed as a OP procedure. But nowadays it is rarely used in practice. It vapourizes tissue to a depth of 5-7mm.Well suited for large, irregular lesions of all grades.

ADVANTAGES:

1. Healing after laser surgery is rapid
2. Minimal side effects including minimal vaginal discharge
3. Less cervical narrowing and a lower stenosis rate
4. No diminution of fertility
5. No obstetric complications
6. Laser does not cause in drawl of SCJ and therefore repeat laser is possible for residual lesion unlike cautery or cryosurgery

DISADVANTAGES:

1. Its acquisition expense.
2. Time required to acquire and maintain laser skills.
3. High maintenance cost of the instrument.

Use of the C02 laser for the management of CIN has decreased dramatically since introduction of LEEP.

LLETZ AND LEEP:

Electro-cautery was used extensively as a destructive procedure. Large Loop Excision Of Transformation Zone and Loop Electrosurgical excision procedure have been used to describe a technique to excise the transformation zone with electro-cautery current. It was described in Europe initially and extensively used in great Britain.

Under paracervical block or local infiltration of cervix with colposcopic guidance, area to be removed is visualised. An appropriate loop size is selected to a depth of 6 - 7mm, extending 4-5mm beyond the affected area.60-80 watt will allow smooth excision and decrease the tissue resistance. After excision of an abnormal cervical tissue, bleeding areas are cauterized with ball electrode using coagulation current and monsel solution.

LEEP:

It is even simpler than LLETZ .LEEP is applicable anywhere in the lower genital tract whereas LLETZ is applicable only to the cervix.

ADVANTAGES:

1. Outpatient procedure
2. It is under colposcopic control and has the advantage of being diagnostic and therapeutic procedure at the same time.

3. Easy to learn, to teach and to apply.
4. Provides tissue specimen for HPE.

DISADVANTAGES:

1. Produces thermal damage.
2. Sometimes causes significant bleeding.
3. Cervical stenosis.
4. Increased risk of preterm labour, preterm premature rupture of membranes, low birth weight in ubsequent pregnancies.
5. Cervical stenosis.

CO2 LASER CONIZATION:

This technique is burdened by need for regional/general anaesthesia. Laser cone surgical specimens may have significant cautery artefacts which make histopathological evaluation cumbersome.

LEEP technique is more efficient, easily mastered and has widespread applicability. It has replaced laser cone.

LEEP technique produces minimal cautery artefact with appropriate power settings.[52]

CONIZATION:

This technique plays an important role in the management of CIN. Traditional cold knife conisation has been used successfully for generations to excise lesions that extends into the endocervical canal or to rule out invasive cancer. Before the advent of colposcopy, it was used to evaluate an abnormal pap results. It is both diagnostic and therapeutic procedure.

INDICATIONS:

1. Colposcopy fails to demonstrate the limits of the lesion
2. SCJ not seen at colposcopy
3. ECC positive for CIN2 OR CIN3
4. Lack of correlation exists between cytology.biopsy and colposcopy results.
5. Suspected microinvasive lesions based on biopsy,colposcopy or cytology results
6. Colposcopist unable to rule out cervical cancer.

Lesions with positive margins recurs after conisation [53,54,55]. Endocervical gland involvement also results in recurrence.[56] LEEP is simpler technique when compared with conization.

LEEP, conization and laser excision showed no difference in recurrence of dysplasia or in pregnancy outcomes by examining the long term effects of all these procedures in a prospective study [57].

COMPLICATIONS:

1. Significant bleeding may occur in the first 24 hrs or 10-21 days after surgery when sutures dissolve.
2. Cervical stenosis occurs in about 3% of patients
3. Size of the cone is related to the risk of premature labour. The larger the cone, the greater is the risk.

HYSTERECTOMY:

It is the last resort for recurrent high grade CIN. In the study of 38cases of invasive cancer, the incidence of significant bleeding, infection and other complications including death is higher in this procedure.

INDICATIONS:

1. Microinvasion
2. CIN3 at the endocervical limits of conization specimen in selected patients.
3. Poor compliance with follow up
4. Other gynaec disorders requiring hysterectomy
5. Histologically confirmed recurrent grades of CIN.

Many different treatment techniques are available for cervical dysplasia. In general, ablative or destructive techniques such as cryotherapy or laser ablation are less injurious to the cervix and have a lower risk of cervical incompetence in future pregnancies.

Excisional techniques such as LEEP provide a specimen for histologic confirmation of the diagnosis. The severity and the size of the lesion together with the desire for future pregnancy and the training of the surgeon and availability of the equipment all help to guide the choice of treatment technique.

COMPARISON OF ABLATIVE TECHNIQUES:

	Cryotherapy	**Laser ablation**
Cost and portability	Cheap	Expensive
Depth of destruction	4-5mm	7mm
Sepsis	Discharge	Nil
Healing	6-8wks	4wks
Post op tz	Indrawn	Seen

COMPARISON OF EXCISIONAL TECHNIQUES:

Characteristics	**Laser conization**	**Cold knife conization**	**LLETZ**	**LEEP**
Admission	OPD	needed	OPD	OPD
colposcope	used	Not used	used	used
anaesthesia	local	GA	local	local
bleeding	slight	++	slight	-
sepsis	nil	+	slight	slight
Scarring and stenosis	7%	26-36%	rare	rare

WHAT CAN BE DONE TO PREVENT CERVICAL CANCER:

1. PRIMORDIAL PREVENTION : Change of behaviour
2. PRIMARY PREVENTION : HPV vaccine
3. SECONDARY PREVENTION : screening

60% of cervical cancer cases will occur in women who have never had a screening test. Will enable to control carcinoma cervix by diagnosing it early in a stage when treatment can give 100% cure rate.

Cervical cancer is particularly well suited for screening because

1. Cause is understood (HPV, especially 16 & 18- 70 - 75% of cancer cervix).
2. Early stage precursors of the disease can be identified(long pre invasive state)
3. Simple outpatient procedure that can treat precursor lesions with a high degree of success.

COMPARISON BETWEEN VIA AND CYTOLOGY:

	Sensitivity(%)	Specificity(%)
CYTOLOGY	29-56	97-100
VIA	76-84	79-83

SCREENING METHODS PERFORMANCE:

Screening Tests Sensitivity

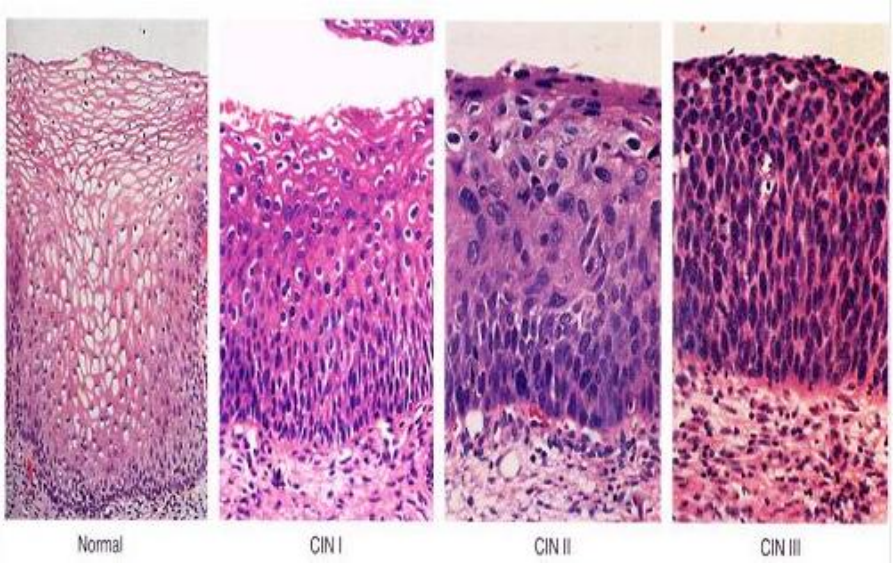
Specificity

Conventional cytology	**44-78%**	**91-96%**
HPV-DNA TESTING	66-100%	61-96%
VIA	67-79%	49-86%
VILI	78-98%	73-93%
COLPOSCOPY	67-74%	65-72%

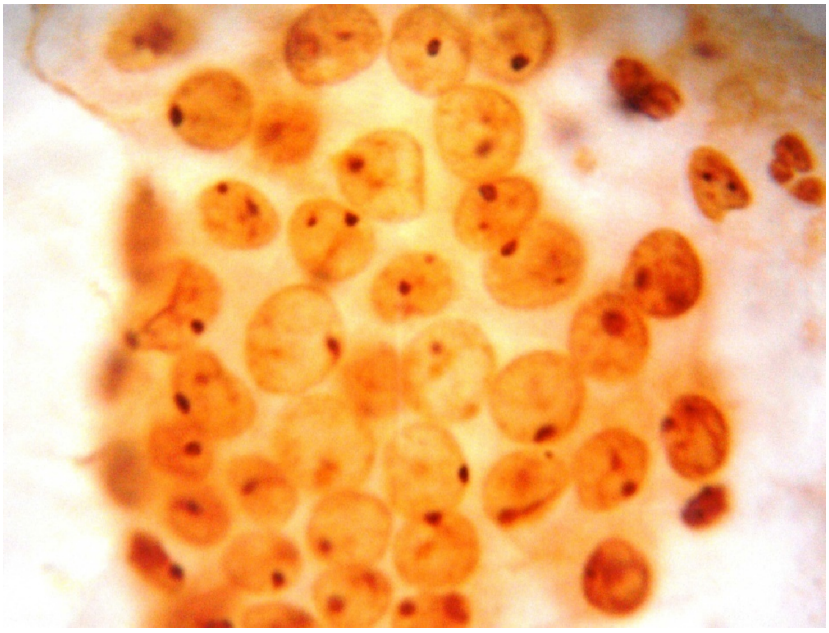
OMANABAD RCT OF CERVICAL SCREENING INDIA

RESULTS OF TREATMENT OF CIN

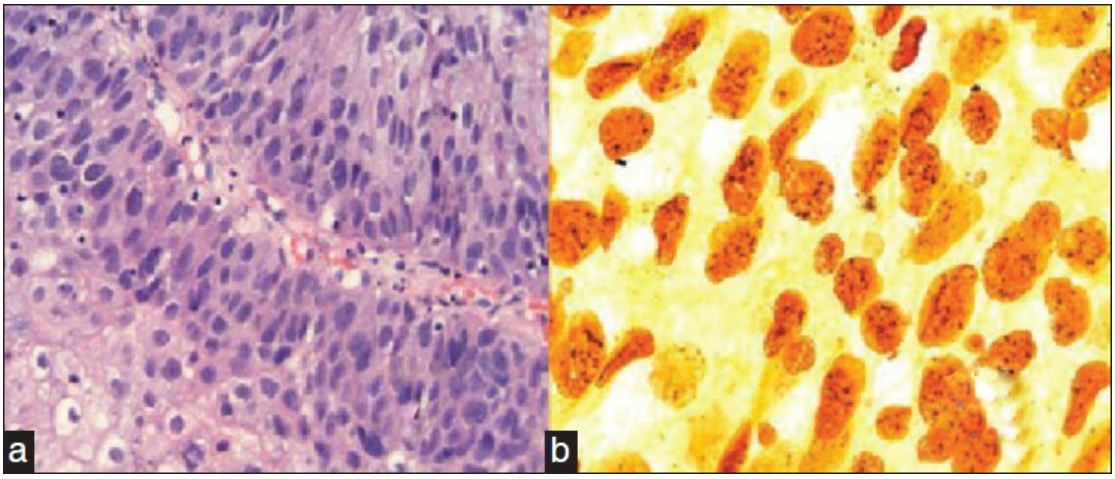
Treatment	Total number	Cured (%)
CRYOTHERAPY	562	477(85%)
LEEP	422	357(85%)



Grades of Cervical Dysplasia

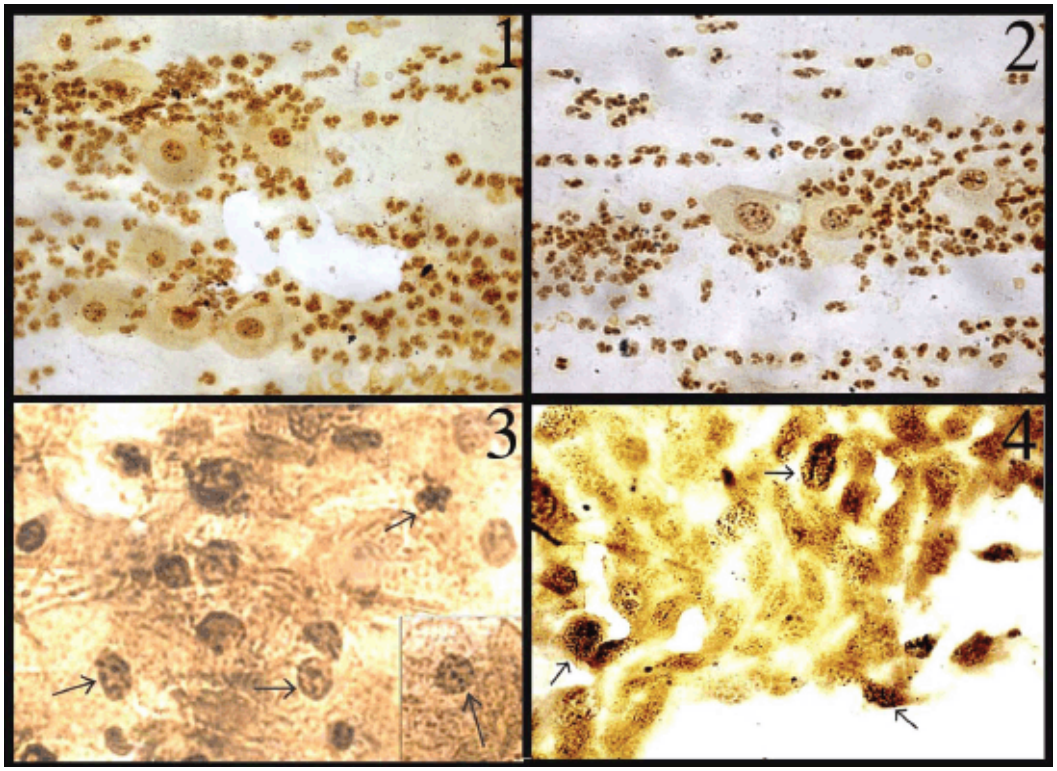


Normal Cervix



a. HPE, b. AgNOR

Squamous Cell Carcinoma



Grades of Cervical Dysplasia in AgNOR Stain

MATERIALS AND METHODS

This study was conducted in our department of obstetrics and gynaecology, Tirunelveli medical college in the period of may 2013 to may 2014. 100 patients from gynaec ward and gnaec OPD were selected after obtaining informed written consent.

INCLUSION CRITERIA:

1. AGE 20-70 yrs
2. Patients with symptoms like profuse white discharge, post coital bleeding, intermenstrual and post menopausal bleeding.
3. Patients with erosion, growth and ulcer diagnosed by speculum examination.
4. Patients with pap smear showing dysplasia

EXCLUSION CRITERIA:

1. Women with age >70 and <20 yrs
2. Pregnant women
3. Women who does not have undergone sexual intercourse
4. Women who underwent hysterectomy or other ablative procedures.

100 patients from gynaec OPD and ward were randomly selected. A detailed history regarding Age, socioeconomic status, educational status, parity, age at marriage, contraceptive usage and obstetric history

were recorded, which was followed by thorough gynaecological examination, cervix biopsy taken from all patients.

TECHNIQUE OF PUNCH BIOPSY OF CERVIX:

After placing patient in lithotomy position, labia were separated and cervix visualised by introducing the sim's speculum into the vagina. Biopsy was taken using punch biopsy forceps, specimen was put in 10%formalin and sent for HPE.

After paraffin embedding, thin sections were made and stained. Apart from the routine H&E stain, AgNOR staining were used.

AgNOR STAINING

MATERIALS FOR STAINING:

1. 5micron thin histopathological sections
2. 50%aquous silver nitrate solution
3. 2%gelatin
4. 1%formic acid

METHODS FOR PREPARING AgNOR STAINING:

Silver stain was used to identify NORs,to evaluate their function and to identify the chromosomes in cytogenetic preparations.

This was first applied by ploton et al (1986), subsequently popularized by crocker et al.

1. Sections were deparaffinised in xylene
2. Hydrated through 100% and 95% ethanol to water
3. Silver stain was prepared by mixing 2%gelatin in 1%formic acid at room temperature
4. Solution was filtered through filter paper
5. One part of solution was mixed with 2 parts of 50%silver nitrate immediately before use.
6. Staining done at dark room temperature for 30-35 min

7. Sections were washed thoroughly in water,dehydrated using 95% and 100%ethanol
8. Sections mounted in a permanent mounting medium.

AgNOR SCORE:

Stained slides were viewed under oil immersion and silver dots are manually counted using light microscope. AgNORs were identified as dark brown/black intranuclear dots. Number of dots were identified in each nucleus.

Clusters of several AgNOR were interpreted as a single unit if the dots are not identified separately.AgNOR score was expressed as mean AgNOR after counting atleast 100 cells.

Two methods to calculate AgNOR score:

1. Counting method
2. Morphometric method

We followed counting method i.e, manual method by carefully focusing through the section thickness at very high magnification. to prevent the counting errors,it was repeated by another person.

RESULTS AND ANALYSIS

Statistical analysis and interpretation:

The study subjects were described in respect of their demographic characteristics in terms of mean and proportions, which ever applicable. Similarly the socio economic and clinical characteristics of them were described in terms of proportions. The above profiles with Histo Pathological Examination were associated by χ^2 (Chi-square) test. The relationship between continues variable more than two groups and AgNOR score were studied by ANOVA and Pearsonic correlation coefficients. The dichotomous attributes and AgNOR score relationships were studied by Students 't' test. The HPE cervical lesions and AgNOR score were studied by ANOVA. The above statistical procedures were performed by the statistical package namely IBM SPSS statistics-20.The $P \leq 0.05$ was considered as statistically significant in two tail test.

Results:

Description of Study Subjects:

The study subjects were described in terms of their demographic, socio economic and clinical features namely age, education, economic status, parity, duration of married life, multiple partner, contraceptive practice, previous screening, STD History, bleeding and white discharge, SE erosion and growth.

Table: 1. Age distribution of cervical lesions women

Age group (years)	No of women	Percentage
20-29	5	5.0
30-39	29	29.0
40-49	27	27.0
50-59	28	28.0
60-69	11	11.0
Total	100	100.0
Mean ± Std deviation	44.8±10.2 years. And range 24-60 years.	

The table 1 describes the age distribution of the women with cervical lesions. Among them the maximum (29%) of women in the age bracket of 30-39 and the least (5%) in the age group of 20-29 years. The other age groups of 40-49 and 50-59 were 27% and 28% respectively. The mean age of them was 44.8±10.2 years.

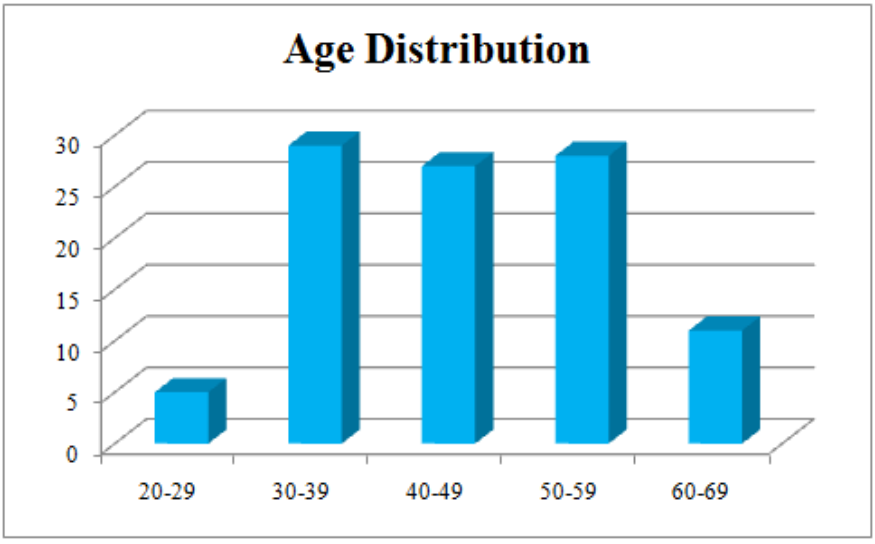


Table: 2. Educational status of study subjects.

Educational Status	No of women	Percentage
illiterate	55	55.0
< 10th Std.	27	27.0
10th and above.	18	18.0
Total	100	100.0

The above table-2 shows the educational status of the study subjects. Majority (55%) of women were illiterates. One fourth (27%) of the women had studied 10th or up to 10th std. The remaining 18% had studied 10th and above std.

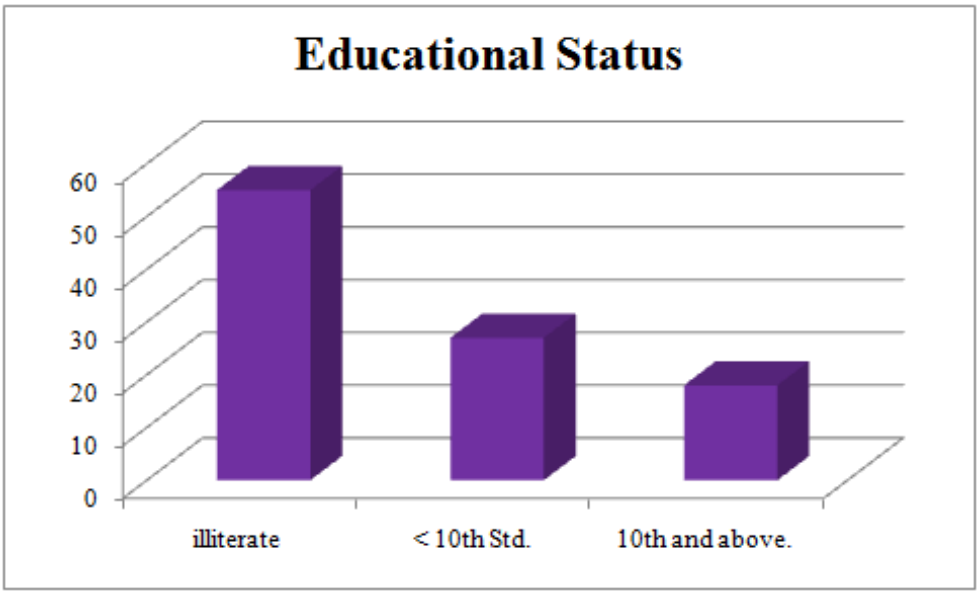


Table:-3. Classification of women according to income status:

Monthly Income Status	No of women	Percentage
<1000	65	65.0
1000-1500	23	23.0
1500-2000	12	12.0
Total	100	100.0

The table 3 states the monthly income of the women. Among them 65% of women had their monthly income Rs. <1000. The remaining 23% and 12% of women had their monthly income in between 1000-1500 and 1500-2000 respectively.

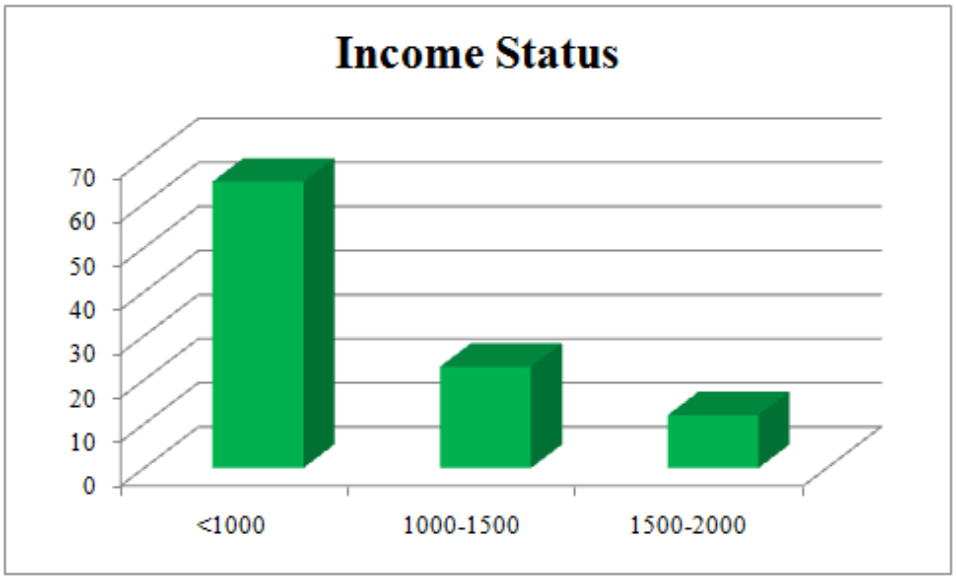


Table: 4 Parity distribution of cervical lesions women

Parity	No of women	Percentage
0	1	1.0
2	34	34.0
3	37	37.0
4	22	22.0
5	6	6.0
Total	100	100.0
Mean ± Std deviation	3.0±0.9	

The table 4 describes the parity level of the mothers. Among them 1% of mothers had no parity. Majority (37%) of mothers had the parity3. The second, fourth and fifth para mothers were 34%, 22% and 6% respectively. The mean parity of the mothers was 3.0±0.9.

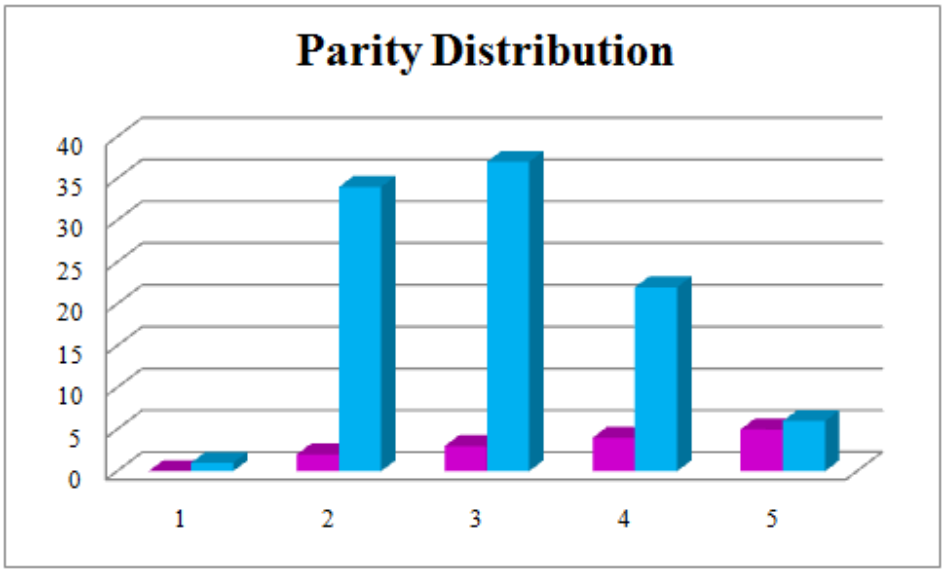


Table: 5 Duration of married life of the study subjects:

Duration of Married life years	No of women	Percentage
<10	14	14.0
10-20	24	24.0
20-30	26	26.0
30-40	26	26.0
40-50	10	10.0
Total	100	100.0
Mean ± Std deviation		

The table 5 states the duration of married life of the women. Out of the above only 10 and 14 percentages of women had their married life <10 years and 40-50 years respectively. The maximum (26%) of women had their married life in 20-30 and 30-40 years each. The remaining 24% had their married life between 10-20 years.

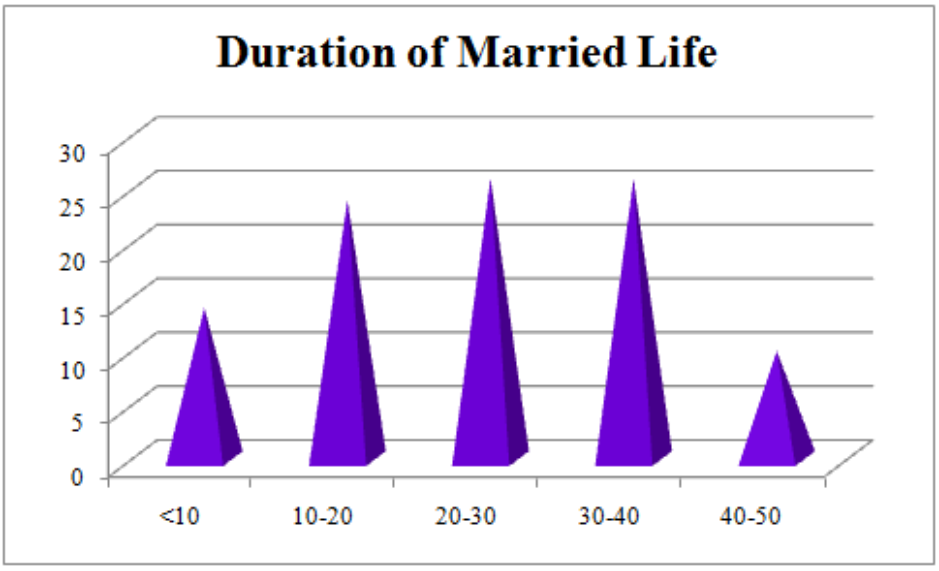


Table: 6. Distribution of Multiple partners of the subjects.

Multiple Partners	No of Women	Percentage.
Nil	99	99.0
Yes	01	01.0
Total	100	100.0

Table 6 states the multiple partners of the study women. Majority (99%) of women did not have multiple partners and only 1% of women had multiple partners.

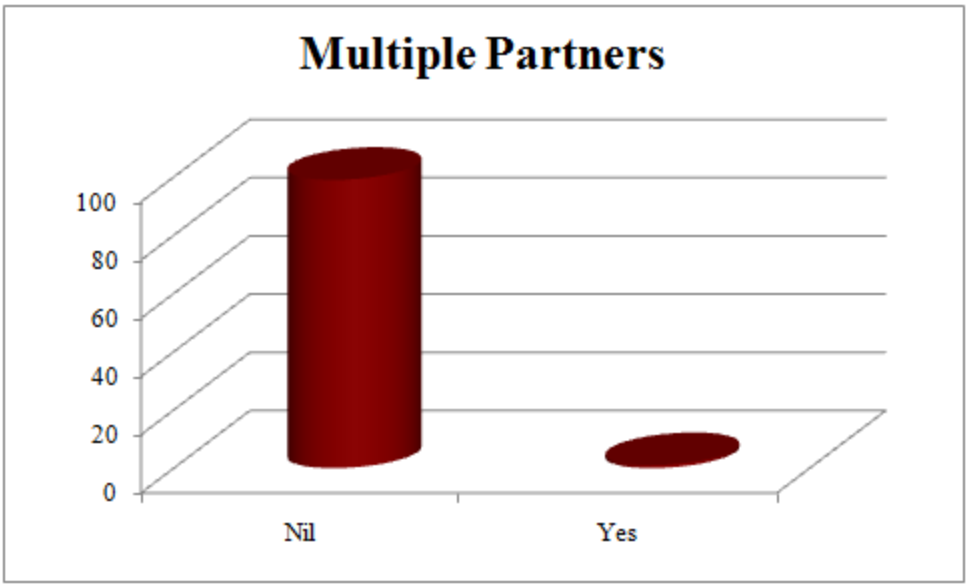


Table:-7. Practice of contraception:

Contraception	No of women	Percentage
Barrier	3	3.0
IUD	11	11.0
Oral Pills	2	2.0
Permanent	55	55.0
NIL	29	29.0
Total	100	100.0

The Table-7 states the contraceptive practice of study women. Among them the maximum (55%) number of women were practicing permanent methods namely tubectomy. Next to that 29% of women were not practicing any one of the methods. The IUD, Barrier and Oral pills were practiced by 11, 3 and 2 percentages respectively.

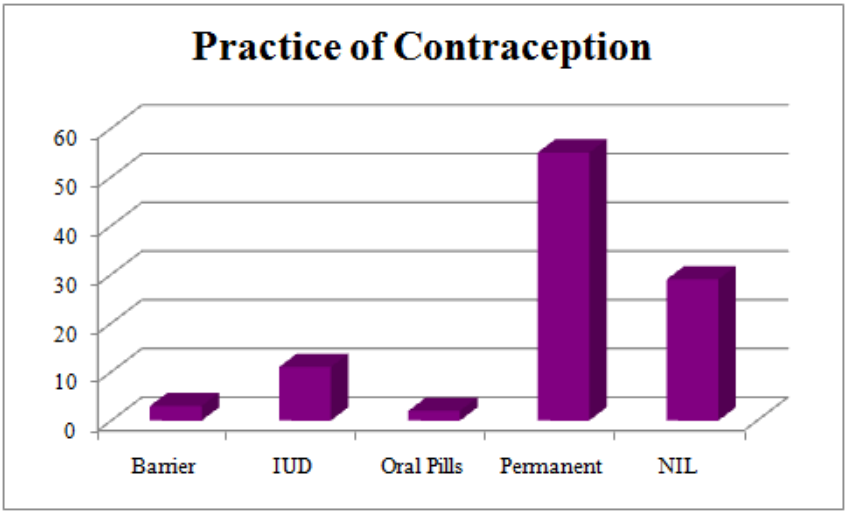


Table: 8. History of previous screening.

Screening history	No of women	Percentage
Nil	93	93.0
Yes	7	7.0
Total	100	100.0

The previous history of screening was found in the above table-8. Among the subjects 93% did not have any history of screening and only 7% had the same.

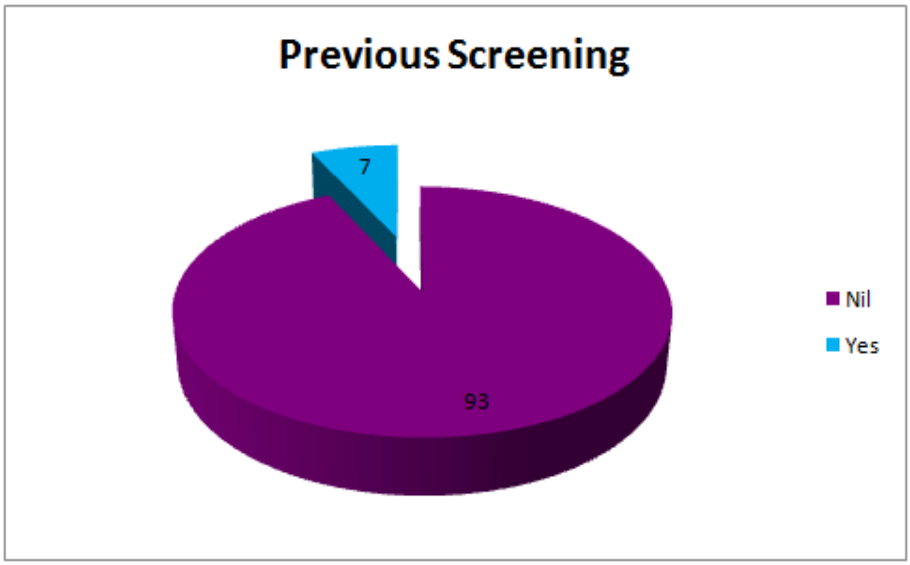


Table:9. Findings of Speculam examination (SE).

SE Findings	Results	No of women	Percentage
Erosion	No	54	54.0
	Yes	46	46.0
	Total	100	100.0
Growth	No	69	69.0
	Yes	31	31.0
	Total	100	100.0

Table-9 reveals that 54% of had no erosion and 46% had it. But the growth was not found in 69% and found in 31% of the study women.

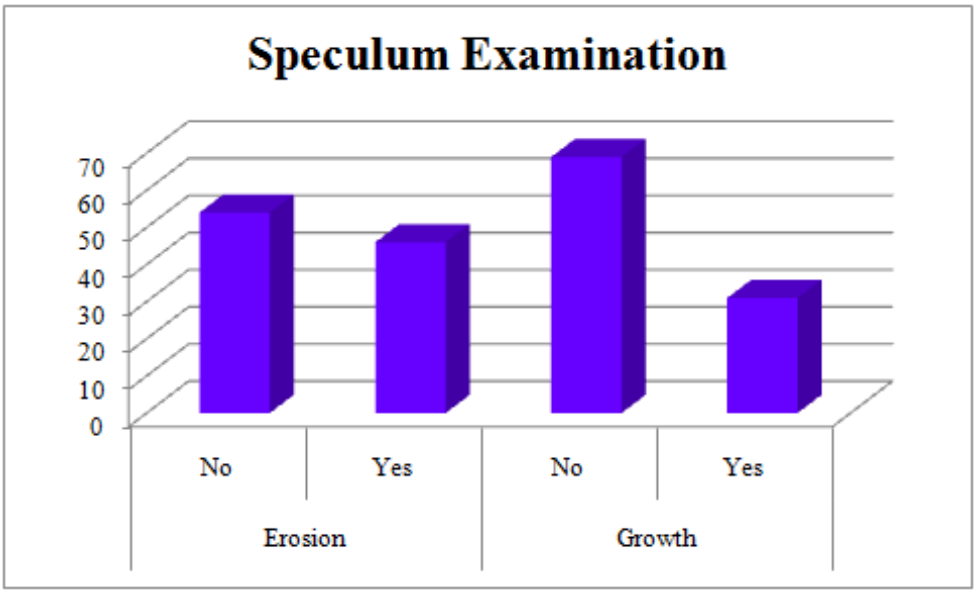


Table:10. Distribution of bleeding, white discharge and both.

Category	Results	No of women n=100	Percentage
Bleeding	Nil	51	51.0
	IMB	8	8.0
	PMB	34	34.0
	PC	7	7.0
White discharge	No	40	40.0
	Yes	60	60.0
Both	YES	14	14.0

The above table 10 states the bleeding and white discharge of the mothers. In respect of bleeding more than half (51%) of the mothers no bleeding and the IMB,PMB and PC had 8%, 34% and 7% respectively. Majority (60%) had bleeding. Only 14% had both.

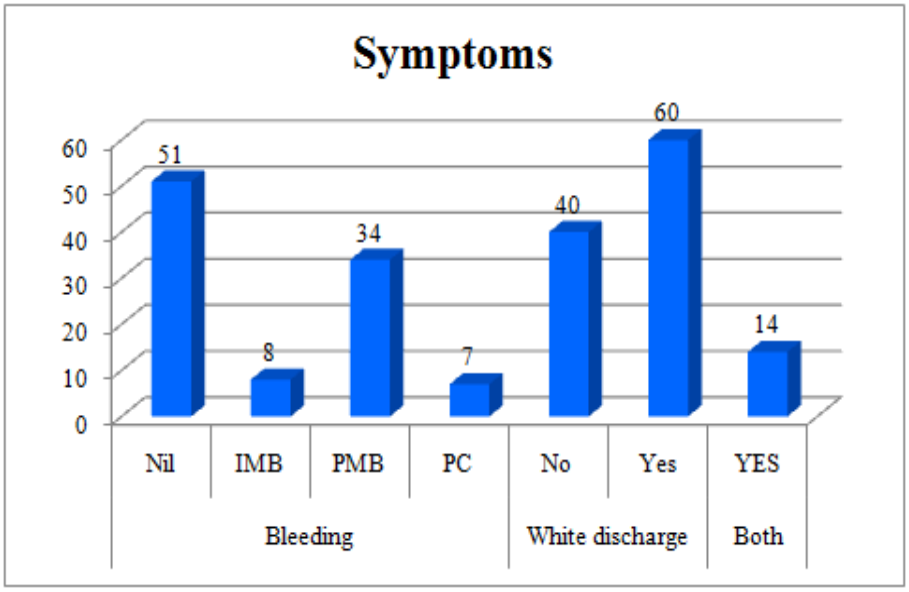
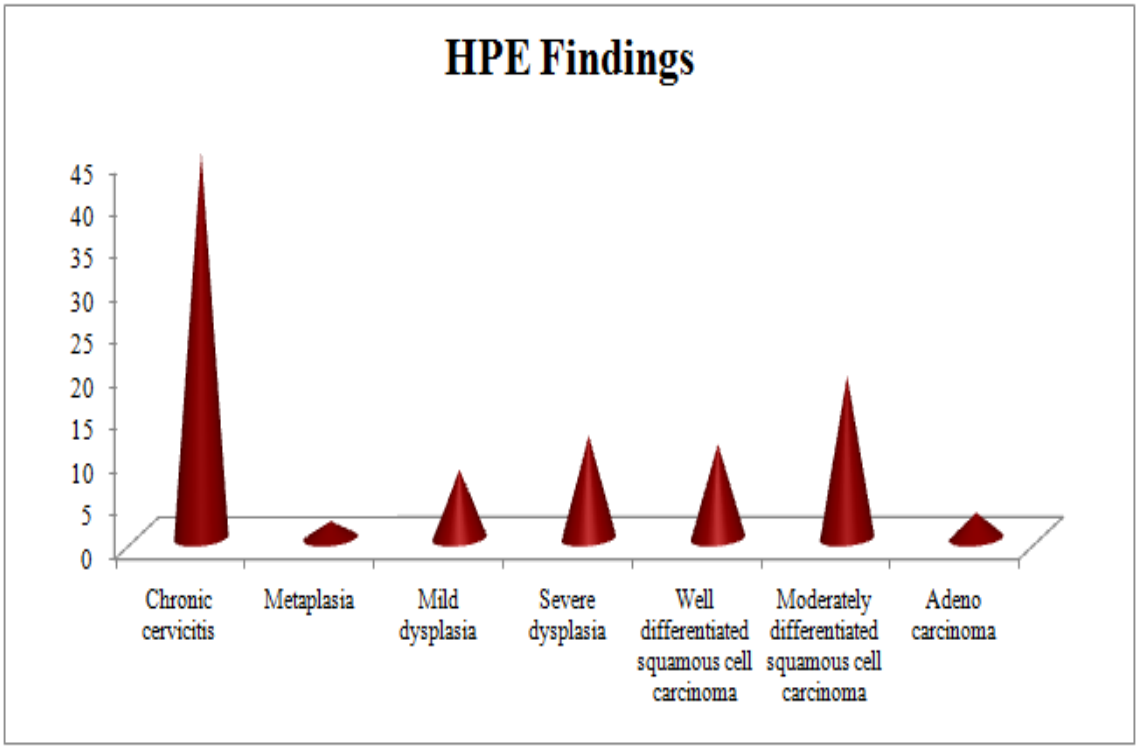


Table: 11 Histopathological examinations of study subjects:

Histopathological examination	No of women	Percentage
Chronic cervicitis	45	45.0
Metaplasia	2	2.0
Mild dysplasia	8	8.0
Severe dysplasia	12	12.0
Well differentiated squamous cell carcinoma	11	11.0
Moderately differentiated squamous cell carcinoma	19	19.0
Adeno carcinoma	3	3.0
Total	100	100.0

The table 11 classifies the study subjects according to their carcinoma characteristics. Nearly half (45%) of them were having Chronic cervicitis and 2% reported with Metaplasia. Mild and Severe dysplasia cases were 8 and 12 percentages respectively. Well and Moderately differentiated squamous cell carcinoma cases were 11 and 19 percentages respectively.


HPE Findings
45
40
35
30
25
20
15
10
5
0
Chronic cervicitis
Metaplasia
Mild dysplasia
Severe dysplasia
Well differentiated squamous cell carcinoma
Moderately differentiated squamous cell carcinoma
Adeno carcinoma

Association between characteristics and HPE findings:

Table:12. Age and HPE findings

Age group	Histopathological findings							Total
	Cervicitis	Metaplasia	Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
20-29	5	0	0	0	0	0	0	5
30-39	17	2	5	1	3	1	0	29
40-49	14	0	3	0	6	2	2	27
50-59	8	0	0	13	2	4	1	28
60-69	1	0	0	5	1	4	0	11
Total	45	2	8	19	12	11	3	100
χ^2	62.271 df=24, P<0.001							

The table 12 associates age and histopathological findings of study subjects. The age and HPE findings strongly associated (P<0.001). That means that incidence of cervical cancer increases according to increase in age of women.

Table:13. Educational status and HPE findings:

Edun. Status	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Illiterate	19	1	4	16	5	8	2	5
<10 Std	16	1	3	1	2	3	1	29
10^{th}& +	10	0	1	2	5	0	0	27
Total	45	2	8	19	12	11	3	100
χ^2	18.98 df=12, P=0.089							

The table 13 states the association between educational status and carcinoma episodes. The results revealed that there was no significant association between the education and cervic cancer episodes of the women (P>0.05).

Table:14. Socio economic status and HPE findings

SE status	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
<1000	25	1	5	15	3	10	3	5
1000-1500	14	1	2	2	4	1	0	29
1500-2000	6	0	1	1	5	0	0	27
Total	45	2	8	19	12	11	3	100
χ^2	22.241,df=12, P<0.001							

The table 14 shows the association between socio economic status of women and Cervical Ca. episodes. The two attributes were significantly associated ($p<0.05$). That means the prevalence among the low economic status women was more than high economic status women.

Table:15. Parity and HPE findings

Parity	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
0	0	0	0	0	0	0	1	5
2	20	2	5	1	6	0	0	29
3	18	0	2	6	4	5	2	27
4	7	0	1	8	2	4	0	28
5	0	0	0	4	0	2	0	11
Total	45	2	8	19	12	11	3	100
χ^2	62.271 df=24, P<0.001							

The table 15 states the association between parity and HPE. The parity was strongly associated with Cervical Cancer (P<0.001). The incidence were increasing according to the increase of parity.

Table:16. Duration of married life and HPE findings

Married life duration	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
0-10	11	0	2	0	1	0	0	5
10-20	15	1	3	1	2	1	1	29
20-30	11	1	3	2	4	4	1	27
30-40	8	0	0	12	4	1	1	28
40-50	0	0	0	4	1	5	0	11
Total	45	2	8	19	12	11	3	100
χ^2	57.121, df=24, P<0.001							

The table 16 states the association between duration of married life and HPE. The duration of married life was strongly associated with Cervical Ca. episodes (P<0.001). The incidence were increasing according to the increase of duration of married life.

Table:17. Contraceptive adoption and HPE findings

Contraception	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Nil	11	1	1	8	4	3	1	29
Barrier	3	0	0	0	0	0	0	3
IUD	7	0	3	0	0	1	0	11
Oral Pills	0	0	1	1	0	0	0	2
Perm	24	1	3	10	8	7	2	55
Total	45	2	8	19	12	11	3	100
χ^2	24.129, df=24, P=0.454							

The table 17 states the association between contraception and carcinoma episodes. The results revealed that there was no significant association between the contraception practice and cervical cancer episodes of the women (P>0.05).

Table:18. Multiple partners and HPE findings

Multiple Partner	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Nil	45	2	8	18	12	11	3	99
Yes	0	0	0	1	0	0	0	1
Total	45	2	8	19	12	11	3	100
χ^2	4.305 df=6, P=0.635							

The table 18 shows the association between multiple partner and carcinoma episodes. The results revealed that there was no significant association between the multiple partner and cervical cancer episodes of the women (P>0.05).out of modesty, only one has accepted multiple partners.

Table:19. Bleeding and HPE findings

Bleeding	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Yes	12	0	2	18	4	11	2	49
Nil	33	2	6	1	8	0	1	51
Total	45	2	8	19	12	11	3	100
χ^2	41.654 df=6, P<0.001							

The table 19 states the association between bleeding and HPE. The bleeding was strongly associated with Cervical Ca. episodes (P<0.001).In all the patients with complaints of bleeding,majority of the patients presented with moderately differentiated carcinoma.(36.7%)

Table:20. White discharge and HPE findings

White discharge	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Yes	36	2	5	6	9	1	1	60
Nil	9	0	3	13	3	10	2	40
Total	45	2	8	19	12	11	3	100
χ^2	29.142 df=6, P<0.001							

The table 20 states the association between white discharge and HPE. The white discharge was strongly associated with Cervical Ca. episodes (P<0.001). In all the patients with complaints of white discharge,majority of the patient presented with chronic cervicitis(60%)

Table:21. Both (white discharge and bleeding) and HPE findings

Both	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Yes	5	0	1	5	1	1	0	13
Nil	40	2	7	14	11	10	3	87
Total	45	2	8	19	12	11	3	100
χ^2	4.249 df=6, P=0.643							

The table 21 shows the association between both and carcinoma episodes. The results revealed that there was no significant association between the both events and cervical cancer episodes of the women (P>0.05).

Table:22. Previous screening and HPE findings

Previous screening	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Yes	3	0	0	1	1	2	0	7
Nil	42	2	8	18	11	9	3	93
Total	45	2	8	19	12	11	3	100
χ^2	3.220 df=6, P=0.781							

The table 22 shows the association between pre screening and carcinoma episodes. The results revealed that there was no significant association between the pre screening and cervical cancer episodes of the women (P>0.05).

Table:23. S/E- Erosion and HPE findings

S/E Erosion	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Yes	30	1	5	1	10	0	0	47
Nil	15	1	3	18	2	11	3	53
Total	45	2	8	19	12	11	3	100
χ^2	39.827 df=6, P<0.001							

The table 23 states the association between SE Erosion and HPE. The S/E- Erosion was strongly associated with Cervical Ca. episodes (P<0.001).

66.6% of patients with chronic cervicitis,62% of patients with mild dysplasia and 84% of patients with severe dysplasia showed erosion cervix in their per speculum examination.

Table:24. S/E- Growth and HPE findings

S/E Growth	Histopathological findings							Total
	Chr. Cervicitis	Metaplasia	Mild Dysplasia	Mod differ SCC	Severe Dysplasia	Well differ SCC	Adeno CA	
Yes	1	0	0	18	0	11	2	47
Nil	44	2	8	1	12	0	1	53
Total	45	2	8	19	12	11	3	100
χ^2	39.827 df=6, P<0.001							

The table 24 states the association between SE Growth and HPE. The S/E showing Growth was strongly associated with Cervical Ca. episodes (P<0.001). 85.7% of invasive cancer presented with friable growth.

Relationship between characteristics and AgNOR scores:

Table:25. Relation between age and AgNOR.

Sl. No	Age group	n	AgNOR		95% CI of mean	'F'	P	Significance between
			Mean	SD				
1	20-29	5	2.8	0.7	1.8-3.7	10.287	P<0.001	The AgNOR Means of 2&3, 4&5 were Not significant. (P>0.05) All AgNOR means between the age groups were statistically significant. (P<0.05)
2	30-39	29	3.4	1.7	2.8-4.1			
3	40-49	27	4.0	1.6	3.4-4.7			
4	50-59	28	5.5	1.8	4.8-6.2			
5	60-29	11	6.1	1.2	5.3-6.9			
	Total	100	4.4	1.9	4.0-4.8			
r=0.550 & P<0.001								

The above table 25 states the relationship between age AgNOR score. The age was increasing; the AgNOR score was also increasing. Both were positively significantly correlated. (r=0.550 and P<0.001).

Table:26. Educational status and AgNOR score.

Sl. No	Education	n	AgNOR		95% CI of mean	'F'	P	Significance between
			Mean	SD				
1	Illiterate	55	4.8	1.9	4.3-5.3	3.031	P=0.053	All were Not Significant (NS)
2	<10th Std	27	3.7	1.8	3.1-4.4			
3	10+ Std	18	4.2	2.0	3.3-5.2			
	Total	100	4.4	1.9	4.0-4.8			

Table 26 shows the relationship between education and AgNOR score. The results revealed that there was no significant relationship between them (P>0.05).

Table:27. Socio Economic status and AgNOR score.

Sl. No	SE status	n	AgNOR		95% CI of mean	'F'	P	Significance between
			Mean	SD				
1	<1000	63	4.7	1.9	4.2-5.2	1.757	P=0.178	All were Not Significant (NS)
2	1000-1500	24	3.9	1.9	3.0-4.7			
3	1500-2000	13	4.2	1.6	3.2-5.2			
	Total	100	4.4	1.9	4.0-4.8			

The Table 27 shows the relationship between Socio Economic status and AgNOR score. The results revealed that there was no significant relationship between them (P>0.05).

Table:28. Relation between Parity and AgNOR score.

Sl. No	Parity	n	AgNOR		95% CI of mean	'F'	P	Significance between
			Mean	SD				
1	0	1	7.1	-	-	6.285	P<0.001	AgNOR Means of 2&3, 3&4, 4&5 were Not Significant. (P>0.05) All other AgNOR means between the parities were statistically significant. (P<0.05)
2	2	34	3.5	1.7	2.9-4.0			
3	3	37	4.5	1.9	3.8-5.1			
4	4	22	5.1	1.7	4.3-5.8			
5	5	6	6.8	0.3	6.4-7.1			
	Total	100	4.4	1.9	4.0-4.8			
r=0.374 & P<0.001								

The above table 28 states the relationship between parity and AgNOR score. The age was increasing; the AgNOR score was also increasing. Both were positively significantly correlated. (r=0.374 and P<0.001).

Table:29. Relation between Duration of Married life and AgNOR score.

Sl. No	Durn (years)	n	AgNOR		95% CI of mean	'F'	P	Significance between
			Mean	SD				
1	0-10	14	3.0	1.0	2.4-3.5	10.294	P<0.001	AgNOR Means of 1&2, 1&3,2&3, 3&4, 4&5 were Not Significant. (P>0.05). All other AgNOR means between the Md life were statistically significant. (P<0.05)
2	10-20	24	3.5	1.6	2.9-4.0			
3	20-30	26	4.4	2.0	3.8-5.1			
4	30-40	26	5.4	1.8	4.6-6.1			
5	40-50	10	6.4	0.5	6.0-6.7			
	Total	100	4.4	1.9	4.0-4.8			
r=0.532 & P<0.001								

The above table 29 states the relationship between duration of married life and AgNOR score. The age was increasing; the AgNOR score was also increasing. Both were positively significantly correlated. (r=0.532 and P<0.001).

Table:30. Relation between Contraception and AgNOR score.

Sl. No	FW Methods	n	AgNOR		95% CI of mean	F	P	Significance between
			Mean	SD				
1	Nil	29	4.8	1.9	2.4-3.5	2.891	P=0.026	AgNOR Means of all except 1&3 were Not Significant. (P>0.05). AgNOR means of 1&3 was statistically significant. (P<0.05)
2	Barrier	3	3.0	0.2	2.9-4.0			
3	IUD	11	4.4	1.2	3.8-5.1			
4	OCP	2	4.8	2.9	4.6-6.1			
5	Perm	55	4.6	1.9	6.0-6.7			
	Total	100	4.4	1.9	4.0-4.8			

Table 30 shows the relationship between contraception and AgNOR score. The results revealed that there was no significant relationship between them except 1&3 (P>0.05).The AgNOR means between Nil and IUD were significantly differed (P<0.05).

The attributes such as multiple partners, previous screening, bleeding, white discharge, Both (bleeding & White discharge), S/E erosion and S/E growth were dichotomous. They were analyzed and interpreted according to their AgNOR score and the results are furnished in table 31.

Table: 31. Relationships between the above attributes and AgNOR score.

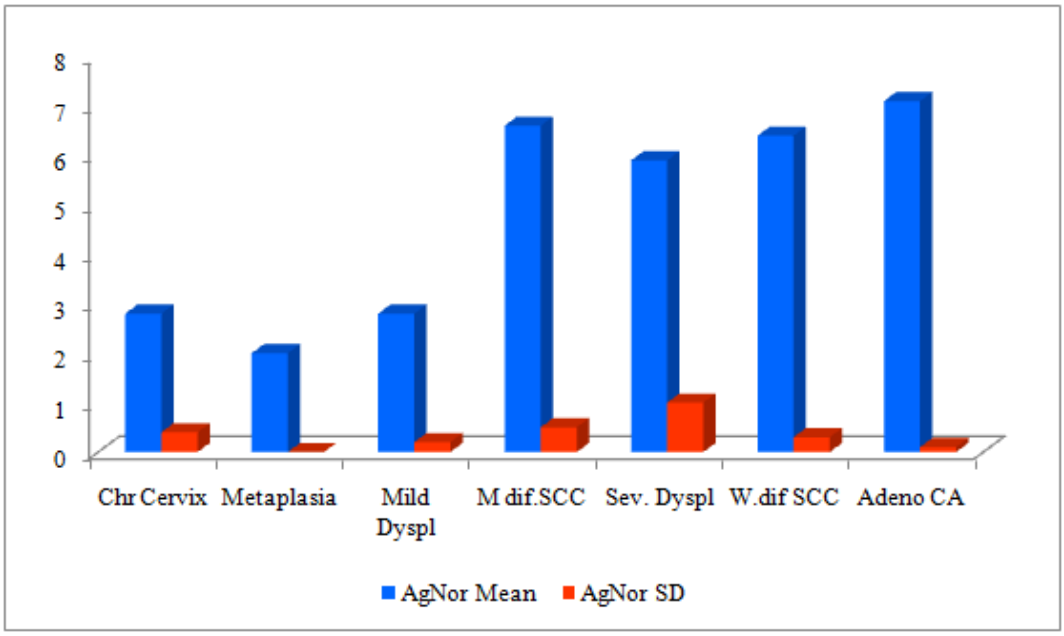
Attributes	Yes of AgNOR		Nil of AgNOR		Differ b/w Means	't'	df	Signifi-cance
	Mean	SD	Mean	SD				
Prev. screening	4.8	1.9	4.4	1.9	0.4	0.578	98	P=0.564
Bleeding	5.4	1.8	3.5	1.5	1.9	5.935	98	P<0.001
White discharge	3.8	1.7	5.4	1.8	1.6	4.368	98	P<0.001
Bld +WD absent	2.9	0.2	4.5	1.9	1.6	1.639	98	P=0.104
S/E erosion	3.5	1.3	5.2	2.0	1.7	4.884	98	P<0.001
S/E growth	6.5	0.8	3.5	1.5	3.0	10.826	98	P<0.001
S/E both absent	3.3	1.8	4.7	1.8	1.4	3.148	98	P=0.002

The AgNOR means of previous screening and not done were not statistically significant (P>0.05). The mean AgNOR score of bleeding subjects was significantly greater than the mean of the patients without bleeding (P<0.001). The mean AgNOR of patients with white discharge was significantly lesser than the mean AgNOR of women without white discharge (P<0.001). But the mean AgNOR of mothers without bleeding and white discharge and either of the complaint mothers mean AgNOR scores were not statistically significant (P>0.05). Similar observation was observed in the case of S/E erosion and growth. But the mean score of both S/E absent (3.3±1.8) was significantly lesser than the either of S/E present mean AgNOR score (4.7±1.8).

Table: 32. Relationship between HPE and AgNOR score.

Sl	HPE	n	AgNOR		95% CI of mean	'F'	P	Significance between
			Mean	SD				
1	Chr Cervix	45	2.8	0.4	2.7-2.9	207.3	P<0.001	AgNOR means of 1&2, 1&3, 2&3, 4&6, 4&7, 5&6, 6&7 were not statistically significant. The other combinations Were statistically significant.
2	Metaplasia	2	2.0	0.0	-			
3	Mild Dysplasia	8	2.8	0.2	2.7-3.0			
4	Mod dif.SCC	19	6.6	0.5	6.4-6.9			
5	Sev. Dysplasia	12	5.9	1.0	5.3-6.6			
6	Well.dif SCC	11	6.4	0.3	6.1-6.6			
7	Adeno CA	3	7.1	0.1	6.8-7.3			
	Total	100	4.4	1.9	4.0-4.8			

The table 32 relates the HPE and AgNOR. various HPE findings exhibit statistically significant difference in AgNOR values. chronic cervicitis, metaplasia and mild dysplasia cases have very low scores and the other cases have higher AgNOR scores. The highest score was present in adeno carcinoma followed by moderately differentiated and then well differentiated carcinoma.



DISCUSSION

Invasive cancer of cervix is considered to be a preventable condition as there is an innumerable screening procedures to detect its preinvasive stage.

A study was conducted in our gynnaec OPD and inpatient between the period from may 2013 to may 2014.women with symptoms of bleeding and profuse white discharge were screened. History in detail and thorough gynaecological examination was done. Cervical biopsy was taken from 100 patients and the sections were stained with conventional H & E stain to study the HPE and with silver stain to study AgNOR scores. AgNOR score was correlated with HPE report.

Regarding age distribution, high incidence of chronic cervicitis was seen in the age group 30-40yrs.mild dysplasia was found to be more common between 30-40yrs.severe dysplasia was maximum in 40-49yrs.moderately differentiated and well differentiated squamous cell carcinoma were maximum in 50-60yrs.

Shiva raj et al in his study showed that mean age of patients with carcinoma was 54yrs.mean age for mild dysplasia was 42.5yrs.mean age for severe dysplasia was 44.2yrs.

Chronic cervicitis, dysplasia and malignancies were more commom among illiterates.55% of the women with abnormal cervical lesions were illiterates. This was attributed to lack of awareness of symptoms and inaccessibility of medical facilities.

Chronic cervicitis, mild dysplasia were associated with low socio economic group. It is because of their poor personal hygiene, early age at first intercourse and they are less likely to screen for cervical cancer.40% of dysplasia and 85% of invasive cancer were seen in low income group.

Regarding parity,our study showed increased incidence of dysplasia and invasive carcinomas among multiparous women.In patients with severe dysplasia,50% were P2,30% were P3,20% were P4 and above.

Among patients with invasive cancer,3% were P2,43% were P3,54% were P2 and above.

Misra et al also showed that women of high parity especially with higher age are more prone to progression to invasive cancer.Satija et al in 2012 showed that women with 3 or > births showed 1.51 increased odds ratio to cancer cervix.

Regarding the duration of married life,it has a distinct rolein the genesis of cervical dysplasia and carcinoma.In our study,incidence of dysplasia was 40% in women with marital life of 5-20yrs.Incidence of Squamous cell carcinoma was about 95%in patients with marital life >20yrs.Kustagi et al demonstated that increase in severity of cervical lesions increase with increase in the duration of sexual intercourse.

International agency for research on cancer in 2002 concluded that use of OCPs for >10yrs increase the incidence of cervical cancer.In our study,13% of mild dysplasia used OCPs.5.2% of patients with moderately differentiated carcinoma gave history of OCP usage.

35% of patients with mild dysplasia used IUCD.55% of patients with mild dysplasia and severe dysplasia were permanently sterilized.

15% of patients with mild dysplasia and 30% of patients with severe dysplasia were not using any methods.

60% of patients with squamous cell carcinoma were permanently sterilized or non users of contraceptive methods.Since our study sample is small,we could not prove whether OCPs increase the incidence of cervical cancer.11% of women with post coital bleeding will present with cervical cancer(shapley et al).45% of patients with cervical cancer presents with bleeding per vaginum.

Early cervical cancer has no symptoms. Bleeding per vaginum, contact bleeding or profuse white discharge may be the presenting feature. The first symptom of cervical cancer is thin watery, blood tinged vaginal discharge(monk et al 2007).

Study conducted by Gundrajkuppam et al in 2011 showed that white discharge was the most common complaint in >50% of patients with malignancy. In our study,50% of patients presented with complaints of white discharge,36% of patients with bleeding and 14% presents with both.

In all the patients with bleeding, majority (35%) presented with moderately differentiated carcinoma. In all the patients with white discharge, majority of patients (60%) presented with chronic cervicitis. Both bleeding and white discharge also more common in 25% of patients with moderately differentiated SCC.

Shapley et al showed that 11% of women with postcoital bleeding will present with cervical cancer.In our study, 7% of patients had post coital bleeding. Among them, 42% of patients had chronic cervicitis,14% had mod. differentiated carcinoma, 28% had well differentiated carcinoma and 14% had severe dysplasia.

Regarding the clinical appearance of cervix,the most common finding ws erosion in patients with chronic cervicitis(63.8%).60% of patients with mild dysplasia and 80% of patients with severe dysplasia presented with erosion cervix.90% of patients with invasive SCC and adenocarcinoma presented with a friable growth.

AgNOR is strictly related to the rapidity of cell proliferation. Therefore, AgNOR can be considered to represent a marker of cell proliferation rate.A number of studies carried out in different tumour types indicates the expression of AgNOR PROTEINS FOUND TO BE GREATER COMPARED TO NON MALIGNANT CELLS. Beningn cells tend to have a regular nucleolus with tight clustering of silver proteins and malignant cells show dispersal of NORs through the nucleus.

Mean number of AgNORs per nucleus was significantly higher in dysplasia(mild-2.8 $\pm$ 0.2,severe-5.9+1.0) and malignant lesions (well differentiated SCC 6.4$\pm$0.3,Mod sifferentiated SCC 6.6+0.5, Adenocarcinoma 7.1$\pm$0.1) as compared to metaplasia(2.0) and chroniuc cervicitis(2.8$\pm$0.4).

All malignant lesions of cervix had significantly Higher AgNOR counts per nucleus compared to mild dysplasia. NOR counts were significantly higher in adenocarcinoma when compared to SCC. There

was a significant difference observed between mean AgNOR counts of chronic cervicitis and dysplasia, mild and severe dysplasia, severe dysplasia and invasive SCC and adenocarcinoma.

Over all mean AgNOR counts were increasing from chronic cervicitis, dysplasia to carcinoma cervix. The following are the major significant AgNOR studies of similar interest which have been carried out in cervix.parallels have drawn between them and present study.

Singh u et al in 2006 found significant AgNOR scores in biopsy specimens.there was a definite increase in the AgNOR score with the progression of lesions i.e.,AgNOR count in CIN1(1.64), CIN2(2.68), CIN3(4.3).

Shivarajet al in 2012,conducted a study which revealed that in cervical pathology, the number and shape of AgNOR dots change from benign to precancerous to malignant tumours. Mean AgNOR dots was 1.55$\pm$0.165 in chronic cervicitis,1.73$\pm$0.257 in metaplasia,1.98$\pm$0.236 in mild dysplasia,2.97+0.38 in moderate dysplasia,4.05$\pm$0.411 in carcinoma respectively.

According to the study carried out by shukla et al in 2013 on 50 cervical smears, it was observed that the AgNOR counts in cervical lesions gradually increases with the severity of the lesions i.e, from a

normal cervix to chronic cervicitis to dysplasia to carcinoma which has the highest score.

Kaushik et al in 2003 carried out a study on cervical biopsies which included 100 cases and categorized them as cervicitis, CIN1, CIN2, CIN3, CA CERVIX. All of the categories in this study showed significant variations in the AgNOR counts($p<0.001$).

Misra JS et al in 2005, conducted a study on 50 cases of cervical smears.In the normal and inflammatory smears ,AgNOR dots ranged from 1-2,LSIL dots ranged from 2-4,HSIL dots 6-8,frank SCC had 10 dots.

Studies	Chronic cervicitis	Metaplasia	Mild dysplasia	Severe dysplasia	Well diff carcinoma	Mod.diff carcinoma	Adeno carcinoma
Prathiba Kuruvilla 1995	-	-	1.8	3	4.3	-	-
Rowlands 1998	-	-	2.2	2.8	-	-	-
Kaushik 1999	2.3	-	3.8	5.1	-	6.4	6.7
Misra 2005	1-2	-	2-4	6-8	8-10	-	-
Singh et al 2006	-	-	1.64 - 2.68	4.3	-	-	-
Shivaraj et al 2012	1.5± 0.165	1.73 ± 0.257	1.98 ± 0.236	2.97 ± 0.387	4.05 ± 0.411	-	-
Sukla et al 2013	2.09	-	2.8	4.17	5.9	-	-

SUMMARY

- 35% of women with chronic cervicitis, 100% of women with metaplasia, 60% of women with mild dysplasia are in the age group 30-39yrs.
- 50% of women with severe dysplasia are in the age group of 40-49yrs.70% of women with well differentiated SCC are in the age group of 50-69yrs.65% of women with mod. Differentiated SCC are in the age group of 50-59yrs.70% of adenocarcinoma are in the age group of 40-49yrs.
- Distribution of cervical cancer was not affected by educational status.
- 55% of chronic cervicitis,40% of dysplasia,80% of carcinoma cervix were reported among low income group.
- Chronic cervicitis, metaplasia, dysplasia were common among P2, where as mod. diff, well diff, adeno carcinoma were common among p3 and above.
- 77% of chronic cervicitis, 85% ofdysplasia and 100% of carcinoma were seen in patients with more than 5yrs of sexual life.

- Adeno CA and chronic cervicitis not associated with OCP usage.only 13% of patients with mild dysplasia and 5.2% of patients with mod diff CA gave H/O OCP usage.
- 80% of patients with chronic cervicitis, 70% of patients with dysplasia and 24% of patients with carcinoma presented with white discharge.
- 24% of patients with chronic cervicitis, 30% of patients with dysplasia,80% of patients with carcinoma presented with bleeding.
- White discharge was the major complaint among the women with chronic cervicitis.bleeding per vaginum was the major complaint among the women with carcinoma cervix.
- 64% of chronic cervicitis,60% of mild dysplasia and 80% of patients with severe dysplasia showed erosion cervix on per speculum examination.
- 90% of patients with invasive cancer presented with friable growth.
- As the age increases,AgNOR score also increases.
- Educational status and socioeconomic status are not significantly rekated to AgNOR score.
- As the parity increases AgNOR score also increases.

- Patients with longer duration of married life have higher AgNOR score than recently married.
- Patients with bleeding have higher AgNOR score (5.4) than those with white discharge.
- Patients with growth on per speculum examination have higher AgNOR score (6.5%) than those with erosion.
- Higher score was seen in adeno CA (7.1+0.1) followed by mod diff CA(6.6+0.5) and then well diff CA (6.4+0.3)

CONCLUSION

- Screening has reduced deaths from cervical cancer. It is the only cancer where we can find it early and treat it .hence earlier diagnosis in adult women is a desirable goal.

- In our present study, mean AgNOR counts in cervical epithelium showed a progressive and statistically significant increase from chronic cervicitis, dysplasia, SCC and adeno CA.As the proliferative capacity of the lesion increases, the malignant potential of the lesion also increases. Hence AgNOR scoring can be used as a reliable indicator of cell proliferation, in turn malignant potential of a lesion.

- This study underscored the diagnostic potential of AgNOR counts especially in delineating between dysplasia and invasive cancer cervix. It is simple, easy to perform, inexpensive and reliable proliferative marker in identifying different grades of cervical lesion.

- It has got prognostic significance also.CIN lesions with low AgNOR count are more likely to regress in comparison to CIN lesions with high AgNOR counts

- Our study indicates that AgNOR technique can be used as an adjunct to routine histopathological examination of lesions of the cervix especially in dysplasia. Eventhough more studies are necessary, our preliminary study indicates the diagnostic importance of AgNOR counts in cervical lesions and other lesions of cervix.

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PROFORMA

Serial No. : Name :

IP/ OP No. : Age :

Income : Education :

I. Complaints

1. Bleeding PV

Post coital bleeding

Continuous / Intermittent

Intermenstrual

Post menopausal

Associated pain

2. Vaginal Discharge

Duration

Quantity

Colour

Foul smell

Pruritis

3. **Lower abdominal pain**

4. **Urinary symptoms**

5. **Bowel symptoms**

6. **Loss of weight**

7. **Loss of appetite**

II. Personal History

Diet

Hygiene

III. Menstrual History

Age at menarche

Cycles

LMP

Age at menopause

IV. Married since

V. Sexual promiscuity

Multiple sexual partners

History of STD

- Husband
- Wife

VI. Obstetrical History

P L A

LCB

Contraception

Barrier

- OCP
- IUCD
- Permanent
- Nil

VII. General Examination

Built	Thyroid
Anaemia	Breast
Pedal edema	BP
Lymphadenopathy	PR
CVS	RS

VIII. Per abdomen

IX. Local examination of Genitalia

Normal

Abnormal

X. Speculum Examination

Cervix

Vagina

XI. Per vaginal examination

XII. P/R

XIII. Biopsy

XIV. AgNOR count

KEY TO MASTER CHART

S.E. Status	-	Socio Economic Status
OCP	-	Oral Contraceptive Pill
Perm	-	Permanent
PC	-	Postcoital Bleeding
PMB	-	Post Menopausal Bleeding
IMB	-	Inter Menstrual Bleeding
Chr. cervicitis	-	Chronic cervicitis
CA	-	Carcinoma
SCC	-	Squamous Cell Carcinoma

MASTER CHART

S.No.	I.P.No	Age	Education	S.E. Status	Parity	Duration of Married Life	Multiple Partners	Contraception	Bleeding	White Discharge	H/O STD	Prev Screening	S/E		HPE	Agnor Count
													Erosion	Growth		
1	51812	40	Nil	<1000	3	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.5
2	55261	34	<10 Std	1001-1500	2	11-20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Mild Dysplasia	3.1
3	55387	53	Iliterate	<1000	3	>20	Nil	Nil	PMB	Yes	Nil	Nil	Nil	Yes	Mod diff SCC	6.9
4	56718	34	<10 Std	<1000	3	5-10	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.08
5	58047	60	Iliterate	<1000	4	>20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	7.2
6	59479	60	Iliterate	<1000	4	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Well diff SCC	6.3
7	60635	50	Iliterate	<1000	4	>20	Nil	Perm	PMB	Yes	Nil	Nil	Nil	Yes	Mod diff SCC	5.6
8	68530	31	< 10 Std	1001-1500	2	5-10	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	1.8
9	58088	55	Nil	<1000	3	>20	Nil	IUCD	PMB	Nil	Nil	Nil	Yes	Nil	Chr cervicitis	2.3
10	60525	60	Nil	<1000	4	>20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	6.8
11	59796	25	<10 Std	1001-1500	2	5-10	Nil	Barrior	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.9

12	64724	56	<10 Std	<1000	4 or >	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Adeno CA	7.1
13	64695	43	<10 Std	1500-2000	3	15-20	Nil	Perm	IMB	Yes	Nil	Nil	Yes	Nil	Severe dysplasia	5.8
14	64706	30	Nil	<1000	2	5-10	Nil	Barrior	PC	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.2
15	64011	48	>10 Std	<1000	3	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.3
16	65135	52	>10 Std	1500-200	3	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	6.1
17	68058	46	Nil	<1000	3	>20	Nil	Nil	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	1.8
18	65718	54	Nil	<1000	2	>20	Nil	Perm	PMB	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	2.2
19	66632	44	< 10 Std	1001-1500	4	>20	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Yes	Chr cervicitis	2.9
20	68078	60	Nil	<1000	4	>20	Nil	Perm	PMB	Nil	Nil	Nil	Yes	Nil	Mod diff SCC	6.6
21	71007	49	>10	1001-1500	3	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Severe dysplasia	5.6
22	73435	59	<10	<1000	4	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.0
23	74678	32	<10	1001-1500	3	5-10	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.8
24	74696	36	>10	<1000	2	15-20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.6

25	79166	24	Nil	100-1500	2	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	1.5
26	76576	51	Nil	<1000	3	>20	Nil	Perm	Nil	Yes	Nil	Nil	Nil	Yes	Mod diff SCC	6.8
27	78388	60	Nil	<1000	4	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.9
28	77428	56	<10	1001-1500	3	>20	Nil	Nil	Yes	Yes	Nil	Nil	Nil	Yes	Mod diff SCC	6.8
29	77329	45	Nil	<1000	3	>20	Nil	Perm	IMB	Yes	Nil	Nil	Yes	Nil	Mild Dysplasia	3
30	72148	36	<10	1500-2000	4	11-20	Nil	IUCD	PC	Nil	Nil	Nil	Yes	Nil	Chr cervicitis	2.5
31	62927	56	Nil	<1000	4	>20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	5.8
32	68865	54	Nil	<1000	5	>20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	6.9
33	68516	52	>10	1500-2000	2	>20	Nil	Perm	Nil	Yes	Nil	Yes	Yes	Nil	Severe dysplasia	5.8
34	68388	50	<10	1001-1500	4	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.9
35	76607	59	Nil	<1000	3	>20	Nil	Nil	PMB	Nil	Nil	Yes	Nil	Yes	Well diff SCC	5.9
36	72308	42	Nil	<1000	3	11-20	Nil	Perm	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	2.6
37	79235	46	Nil	1001-1500	2	>20	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Severe dysplasia	5.3

38	68817	52	Nil	<1000	3	>20	Nil	Perm	PMB	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.9
39	68900	47	<10	<1000	3	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.1
40	76822	49	>10	1001-1500	3	>20	Nil	Perm	Nil	Yes	Nil	Yes	Yes	Nil	Chr cervicitis	2.8
41	76814	35	<10	1001-1500	2	11-20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Metaplasia	2
42	78312	48	Nil	<1000	3	>20	Nil	Nil	IMB	Nil	Nil	Nil	Nil	Yes	Adeno CA	7.2
43	73250	48	<10	<1000	3	>20	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Mild Dysplasia	2.7
44	75755	36	>10	1001-1500	2	11-20	Nil	Perm	PC	Nil	Nil	Nil	Nil	+	Mod diff SCC	7.0
45	77570	43	Nil	<1000	3	>20	Nil	Nil	-	Yes	Nil	Nil	+	-	Chr cervicitis	2.8
46	76812	30	>10	1500-2000	2	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	1.9
47	77164	35	Nil	<1000	2	5-10	Nil	Perm	IMB	Nil	Nil	Nil	Yes	Nil	Chr cervicitis	2.9
48	75720	39	<10	<1000	3	11-20	Nil	Perm	IMB	Nil	Nil	Nil	Nil	Nil	Chr cervicitis	2.5
49	75663	37	Nil	<1000	2	11-20	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Metaplasia	2
50	71076	40	Nil	<1000	4	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Well diff SCC	5.8

51	74679	37	>10	1500-2000	2	11-20	Nil	Perm	Nil	Yes	Nil	Yes	Nil	Nil	Chr cervicitis	3.0
52	76968	60	Nil	<1000	4	>20	Nil	Perm	PMB	Yes	Nil	Nil	Nil	Yes	Mod diff SCC	6.2
53	74054	45	Nil	<1000	3	>20	Nil	Nil	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.5
54	76944	55	Yes	<1000	3	>20	Nil	IUCD	IMB	Nil	Nil	Nil	Nil	Yes	Well diff SCC	6.3
55	74787	34	>10	1001-1500	2	11-20	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	3.2
56	73543	40	<10	1001-1500	2	5-10	Nil	Perm	IMB	Nil	Nil	Nil	Yes	Nil	Chr cervicitis	2.8
57	73558	40	Nil	<1000	4	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Mild Dysplasia	2.9
58	73558	50	Nil	<1000	3	>20	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	2.8
59	73082	50	Nil	<1000	3	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	6.9
60	73102	35	>10	1500-2000	2	5-10	Nil	Perm	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	3.0
61	70936	42	Nil	<1000	3	>20	Nil	Perm	Nil	Yes	Nil	Yes	Yes	Nil	Chr cervicitis	2.8
62	75923	40	<10	1001-1500	3	>20	Nil	Nil	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.9
63	73041	35	<10	<1000	2	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Yes	Nil	Mild Dysplasia	2.5

64	76688	39	Nil	<1000	2	11-20	Nil	Nil	PC	Nil	Nil	Nil	Yes	Nil	Severe dysplasia	5.8
65	78952	27	<10	1001-1500	2	5-10	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Nil	Chr cervicitis	2.9
66	72284	34	Nil	<1000	2	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Nil	Nil	Mild Dysplasia	2.7
67	79983	60	Nil	<1000	4	>20	Nil	OCP	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	6.9
68	79629	40	>10	<1000	3	11-20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.9
69	79720	39	Nil	<1000	3	11-20	Nil	Perm	IMB	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	2.7
70	77678	50	Nil	<1000	4	>20	Nil	Nil	Nil	Yes	Nil	Nil	Yes	Nil	Severe dysplasia	5.8
71	78182	47	Yes	>1000	3	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Well diff SCC	6.4
72	78232	48	<10	1001-1500	2	>20	Nil	Nil	PMB	Nil	Nil	Nil	Yes	Nil	Severe dysplasia	5.7
73	78234	60	Nil	<1000	3	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Well diff SCC	6.3
74	78033	53	Nil	<1000	4	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Well diff SCC	6.7
75	79147	40	>10	<1000	2	11-20	Nil	Perm	PC	Nil	Nil	Nil	Yes	Nil	Chr cervicitis	2.9
76	70854	60	Nil	<1000	5	>20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	Well diff SCC	6.3

77	70675	33	>10	>1500	2	5-10	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Severe dysplasia	5.8
78	70856	40	Nil	<1000	3	11-20	Nil	Perm	Nil	Yes	Nil	Nil	Nil	Nil	Adeno CA	7.0
79	70241	57	<10	<1000	3	>20	Nil	Nil	PMB	Yes	Nil	Nil	Nil	Yes	Well diff SCC	6.9
80	70529	55	Nil	<1000	4	>20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	6.0
81	70805	60	Nil	<1000	4	>20	Nil	Perm	PMB	Nil	Nil	Nil	Yes	Nil	Severe dysplasia	5.4
82	70795	36	>10	1500-200	2	11-20	Nil	IUCD	Nil	Nil	Nil	Nil	Yes	Nil	Mild Dysplasia	2.8
83	73001	53	Nil	<1000	5	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	7.0
84	73556	24	<`0	1500-2000	2	5-10	Nil	Nil	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.2
85	75128	58	Nil	<1000	4	>20	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	3.4
86	74050	33	Nil	1001-1500	2	5-10	Nil	Barrior	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	2.9
87	74092	60	Nil	<1000	5	>20	Nil	Perm	PC	Nil	Nil	Yes	Nil	Yes	Well diff SCC	6.4
88	71186	34	<10	1001-1500	2	11-20	Nil	IUCD	Nil	Nil	Nil	Nil	Yes	Nil	Chr cervicitis	3.2
89	75080	37	>10	1001-1500	2	11-20	Nil	Perm	Nil	Yes	Nil	Nil	Nil	Nil	Sq.Metaplasia	9.2

90	79090	38	Nil	<1000	4	>20	Nil	Perm	PC	Nil	Nil	Nil	Nil	Yes	Well diff SCC	6.6
91	79141	50	Nil	<1000	5	>20	Yes	Nil	PMB	Yes	Nil	Yes	Nil	Yes	Mod diff SCC	7.1
92	79524	35	>10	1500-2000	2	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	2.9
93	79526	50	Nil	<1000	3	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	7.1
94	79743	24	<10	1000-1500	2	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.3
95	79165	55	Iliterate	<1000	4	>20	Nil	Nil	PMB	Yes	Nil	Nil	Yes	Nil	Chr cervicitis	3.4
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97	78016	35	Nil	<1000	2	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Nil	Nil	Chr cervicitis	2.9
98	75918	49	Nil	1500-2000	3	>20	Nil	Perm	Nil	Yes	Nil	Nil	Yes	Nil	Severe dysplasia	5.8
99	75082	55	Nil	<1000	5	>20	Nil	Perm	PMB	Nil	Nil	Nil	Nil	Yes	Mod diff SCC	6.9
100	76985	30	Nil	1001-1500	2	5-10	Nil	OCPS	Yes	Nil	Nil	Nil	Nil	Nil	Mild Dysplasia	2.8